DETERMINATION OF COCAINE AND ITS METABOLITES IN SPECIMENS OF NEONATAL AND MATERNAL ORIGIN

By

RUTH ELLEN WINECKER

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1996

I dedicate this work to my husband, John. See, I told you we would make it TOGETHER!

ACKNOWLEDGMENTS

I would like to thank my doctoral committee. First and foremost, I would like to thank Roger L. Bertholf, Ph.D., for his guidance and enthusiasm during the past four and a half years! He introduced me to the world of clinical chemistry and toxicology with its mix of medicine and analytical chemistry, which I have found to be rewarding, exciting, and fulfilling. For this, I will be forever grateful.

Thanks also go to Ian Tebbett, Ph.D., for allowing me to work on his cocaine project and without which this dissertation would not be possible.

To Bruce A. Goldberger, Ph.D., and Catherine Hammett-Stabler, Ph.D., thanks for assuming the roles of "Honorary Gainesville Mentors;" you provided me with invaluable advice, you were always there when I needed you, and your guidance has been right on target!

Thanks also go to Richard A. Yost, Ph.D., for keeping me on the analytical straight and narrow.

My appreciation also goes to the following folks: Dr. Goldberger's lab group; in particular Jeri Ropero, Dr. Diana Garside, Abraham Tsadik, and Zafer Sabawi for all their help and encouragement; Dr. Tebbett's lab group, in particular, Diane Phillips and Siya, for teaching me HPLC.

My gratitude to Robert C. Hart, John's boss, for allowing John the time off from work to help me.

Finally, I would like to thank the National Institutes of Health, and the Division of Sponsored Research at the University of Florida for funding my research.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

DETERMINATION OF COCAINE AND ITS METABOLITES IN SPECIMENS OF NEONATAL AND MATERNAL ORIGIN

By

Ruth Ellen Winecker

December, 1996

Chairperson: Dr. Roger L. Bertholf
Major Department: Pathology

The increased use of cocaine by women of childbearing age has left many health care scientists searching for improved methods of detecting prenatal cocaine exposure. To that end, a study of the determination of cocaine and its metabolites in various specimens of maternal and neonatal origin was undertaken. Urine (n=75), meconium (n=74), maternal hair (n=66), umbilical cord tissue (n=70), amniotic fluid (n=32) and colostrum (n=21) were collected from pregnant subjects admitted to labor and delivery at Shands Hospital at the University of Florida (Gainesville, FL). Drug use status was ascertained in a confidential maternal interview. Subjects reporting cocaine use were designated as target subjects and those denying use as control subjects.

Meconium and urine specimens were screened by immunoassay for the cocaine metabolite, benzoylecgonine. All specimens were subjected to solid-phase extraction and analyzed for cocaine and its metabolites by high performance liquid chromatography (HPLC) and/or gas chromatography/mass spectrometry (GC/MS). The percentage of positive specimens for each subject type is presented in Table A-1.

Table A-1. Percentage of positive specimens in target and control subjects.

Subject	Hair	Colostrum	Amniotic Fluid	Meconium	Urine	Cord Tissue
Target (%)	73	60	54	50	48	43
Control (%)	12	0	11	9	9	2

Statistical analysis was undertaken to determine if any of these specimens is more likely to detect prenatal cocaine exposure. Results indicate that hair is more likely than umbilical cord tissue to detect prenatal cocaine exposure, but was equal in ability to all other specimens. Moreover, maternal hair may have a greater ability to detect prenatal cocaine exposure but, more studies, with larger numbers of subjects, are needed for results to attain statistical significance. In conclusion, this study has shown that no single specimen is ideal for the identification of cocaine exposed infants for the purposes of classification as exposed or non-exposed in health outcome research. This will continue to be a major stumbling block in studies investigating the adverse effects of prenatal cocaine exposure.

CHAPTER 1
INTRODUCTION

Cocaine is the principal alkaloid obtained from leaves of the coca tree, *Erythroxylon coca*, and is the only known naturally occurring local anesthetic. Archaeologists and historians have chronicled the use of the coca tree for medicinal, religious, and stimulatory purposes at least 15 centuries.[1] Unlike the ancient Incas, who chewed the coca leaves with ash achieving a slow absorption of cocaine, today's user is more likely to ingest more concentrated and highly addictive forms of the drug, such as cocaine powder or crack cocaine.[1,2] In fact, modern forms of cocaine use produce a more intense "high" and a stronger "craving" for repeated use.[1,3] While cocaine was introduced to the United States as a general anesthetic and used in some wine and soft drink preparations during the early to mid 1800s, it became clear by 1900 that the use of cocaine could have serious adverse side effects, including a high potential for abuse. Today cocaine is classified as a Schedule II drug, recognized for its high abuse potential and limited medical usefulness.[1]

Despite increased regulation of its use and a highly publicized governmental "war on drugs," the abuse of cocaine, especially the preparation

known as crack, continues to increase.[4, 5] "Crack" is a freebase form of cocaine that can be smoked because of its stability at temperatures required for vaporization, and is sold in vials that contain one or more "rocks." Crack cocaine first appeared in the United States in 1986 and brought with it a significant increase in the average dose, resulting in an increase in cocaine related morbidity and mortality.[6] The low price, ease of use, and availability have contributed to what many researchers characterize as an explosion of cocaine use in the last decade.[3, 7] The intense euphoria within one minute of smoking crack, followed by the swift inactivation and elimination of the drug from circulation, may contribute to its highly addictive nature and explain the trend away from casual use toward abuse. In fact, while the number of current users (current use is defined by the Substance Abuse and Mental Health Services Administration (SAMHSA) as use of the drug within the past thirty days) of cocaine has declined from 5.8 million in 1985 to 1.9 million in 1991, the number of daily users has increased from 246,000 to 336,000 over the same period.[2,8]

Importantly, drug usage surveys indicate that the heaviest users of cocaine are between 18 and 30 years of age, which in females, are peak childbearing years.[9, 10, 11] The lifestyle of many of these young adult female cocaine abusers includes drug binges in "crack houses," where prostitution is also prevalent.[9,12] Naturally, this has resulted in an increasing number of infants being exposed to cocaine in utero. Estimates of the prevalence of cocaine

abuse include the National Pregnancy and Health Survey which reported that 1.1% of pregnant women from 52 rural and urban hospitals admitted to using cocaine.[13] Self-report surveys underestimate the true exposure rate, as many women are afraid to answer questions concerning drug use truthfully, for fear of social and/or legal repercussions. In fact, when maternal urine specimens were tested, more than twice as many were positive for the cocaine metabolite benzoylecgonine than those who admitted to using cocaine.[14] Estimates based on laboratory screening of mothers and infants for cocaine use range from a low of 1% in a relatively low risk population to a high of 37% in an inner city Chicago population.[15,16]

Pharmacology, Metabolism, and the Addictive Nature of Cocaine

The addictive nature of cocaine can be linked to specific neurotransmitter systems in the brain. The euphoria and excitability that accompany cocaine use are the result of cortical stimulation and increased neurotransmission in the brain's reward pathways.[7,17-20] Some of cocaine's effects are attributed to its inhibition of dopamine and norepinephrine uptake in the synapse. The resulting catecholamine surplus exerts its effect on the sympathetic nervous system causing vasoconstriction, hypertension, and an increase in heart rate.[2,7,18-20] Additionally, cocaine blocks uptake of the inhibitory neurotransmitter serotonin

and its precursor tryptophan hydroxylase, resulting in an acute depletion of serotonin.[21] The combined effects of catecholamine and serotonin surplus is believed to be a key factor in the production of cocaine euphoria but is certainly not the entire process. Despite an enormous amount of research, the exact nature of cocaine produced euphoria is still undefined, and may involve multiple actions of the noradrenergic, dopaminergic, serotonergic, and cholinergic pathways.[1,2,7,17-20]

Cocaine's effects display a biphasic pattern. In addition to the acute euphoria following the administered dose, there is a chronic dysphoria occurring once plasma cocaine levels begin to fall. Cocaine's effects appear to be mediated by the brain's reward centers, specifically the mesolimbic and mesocortical dopaminergic pathways. These pathways are believed to be the basis of addictive use patterns and relapse of cocaine use in individuals who have previously undergone cocaine dependency treatment.[3,21] Cocaine's powerful influence on the brain's reward centers is evident in studies where animals, given unlimited access, preferred cocaine over food, water, and mating. In some cases, self administration continued beyond toxic doses until death resulted.[7,17-19,21]

The three most common routes of cocaine administration are insufflation ("snorting") of cocaine hydrochloride (powder), smoking of crack, and intravenous (IV) injection. Adsorption kinetics are similar for smoking and IV

use, each producing an almost instantaneous high and peak plasma concentrations within five minutes of administration.[2,7,18,19,22] Peak plasma levels by smoking or intravenous routes, after a typical experimental dose of 30-40 mg have been found in the range of 500-2500 ng/mL.[6,18,19] Insufflation results in a much slower rise in plasma drug concentrations with peak levels occurring within one hour. Peak plasma levels following insufflation are also lower than both smoking and intravenous routes, and are roughly 100-500 ng/mL.[2,6,18,19] This is partly due to the vasoconstrictive effects of cocaine in the mucus membranes, which decreases blood flow to the site of application, thereby slowing drug uptake.[1,2,22] Peak concentrations during periods of heavy use, on the other hand, are probably much higher, especially in crack smokers, where the amount of cocaine consumed is measured in multiple gram quantities rather than milligrams.[23] This has not been thoroughly investigated as ethical and safety considerations prohibit administration of large and frequent doses.[18,19,23]

Once ingested, cocaine is rapidly inactivated by the hydrolysis of one or both of the ester linkages, and is eliminated quickly from the body with a half-life of less than an hour.[1,2,18,19] Approximately, 75-90% of a cocaine dose is hydrolyzed, either chemically or enzymatically, by plasma and liver esterases to the water soluble metabolites benzoylecgonine (BE) and ecgonine methyl ester (EME). A majority of ecgonine methyl ester is produced through the action of hepatic cocaine carboxylesterases, but in experiments involving hepectomized

pigs, ecgonine methyl ester production is not entirely eliminated.[24] In earlier experiments, pigs given plasma esterase inhibitors showed no change in ecgonine methyl ester production.[25] Clearly, the production of ecgonine methyl ester is complex, involving more than just one organ and enzyme system. Similarly, benzoylecgonine was once thought to be produced solely by spontaneous hydrolysis of the methyl ester group.[18,19] But benzoylecgonine now known to be produced by the action of plasma cholinesterases and the same hepatic carboxylase that is responsible for the production of cocaethylene.[26]

Until recently, it was generally thought that benzoylecgonine and ecgonine methyl ester were produced and excreted in approximately equal amounts. However, the case has proved to be much more puzzling than originally believed and the ratio apparently dependent on the route of administration.[27,28] In one study cocaine was administered intranasally, resulting in benzoylecgonine concentrations 50% higher than after intravenous or smoking routes. This is thought to be the result of metabolism of cocaine during its passage through the mucosa.[27] In another study, ecgonine methyl ester concentrations amounted to less than 5% of benzoylecgonine levels in plasma after smoking and intravenous routes of administration. Low plasma ecgonine methyl ester levels are apparently the result of rapid conversion to ecgonine by chemical hydrolysis of the methyl ester group.[28]

N-demethylation of cocaine in hepatic microsomes produces norcocaine and further metabolism yields *n*-hydroxynorcocaine, norcocaine nitroxide, and benzoylnorecgonine.[27,29] Norcocaine is rarely detected in plasma because very little is produced during normal metabolism. However, in urine it is often found in small amounts due to the concentrating effect of the kidney.[27] When cocaine is used simultaneously with ethyl alcohol, transesterification by liver carboxylesterases produces cocaethylene. Cocaethylene may also be produced by the action of fatty acid ethyl synthetase, which may use cocaine as a substrate to esterify ethyl alcohol to the methyl ester group.[30] This enzyme has a wide distribution in the body and may contribute to cocaethylene production in the brain, where cocaethylene exhibits pharmacological activity that is longer lasting and more potent than cocaine.[31] Cocaethylene can further be metabolized to benzoylecgonine, norcocaethylene, and ecgonine ethyl ester.[27,31]

Recently, a crack pyrolysis product, anhydroecgonine methyl ester, has been identified in the urine of crack cocaine smokers. This material is formed as a result of thermal breakdown as the crack is smoked and is of interest to researchers looking to distinguish among the different routes of cocaine administration.[27] Another minor metabolite, *m*-hydroxybenzoylecgonine, has been found in significant amounts in meconium, the neonates first bowel movement.[13,32] *M*-hydroxybenzoylecgonine is formed when benzoylecgonine is hydroxylated in the meta position of the benzoyl ring and is of interest because it

holds promise for improving detection of prenatal cocaine exposure. Figure 1-1 depicts cocaine and the current understanding of its metabolism.

The altered metabolic state of pregnant women means that the metabolism of cocaine could be modified in these individuals. Pregnant women are known to have reduced cholinesterase activity, which could slow the metabolism of cocaine, increase the duration of its action and force metabolism through alternative pathways.[18,19] Additionally, the cardiovascular effects of cocaine seem to be more pronounced during pregnancy.[33] Evidence for this includes an increase in the metabolism of cocaine to norcocaine and an increase in sensitivity of alpha-adrenergic receptors, both of which appear to be related to the effects of progesterone.[33,34]

The fetus and neonate may also exhibit alterations in the proposed metabolism of cocaine, because activity of plasma cholinesterases and expression of liver xenobiotic metabolism does not occur until late in gestation.[13,35,36] Experiments indicate that the fetal liver has a very limited ability to metabolize cocaine or other xenobiotics. The first mixed function oxidase system that develops in the fetus is thought to be the cytochrome P-448 enzyme system.

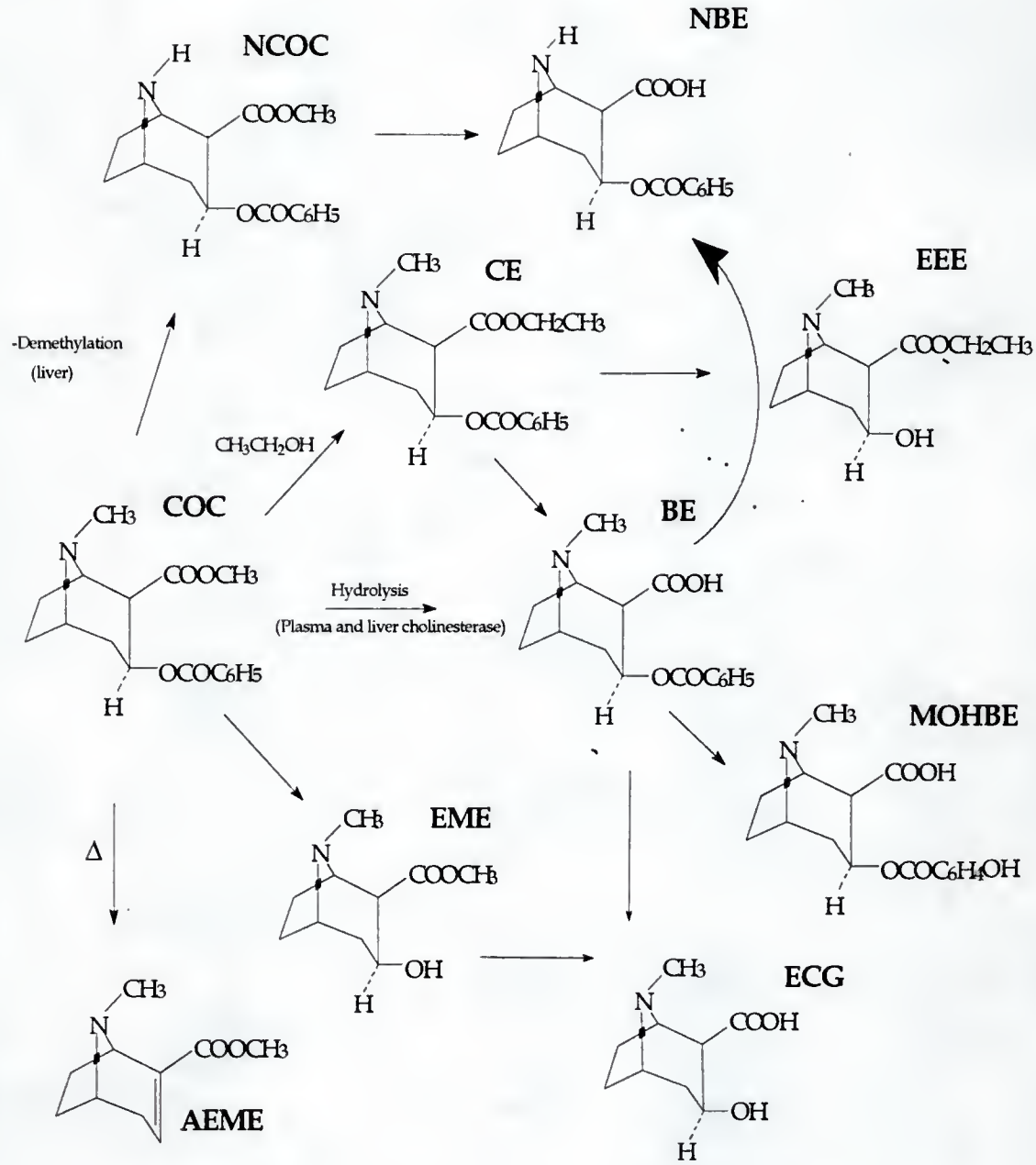


Figure 1-1: Cocaine, its Metabolites, and Crack Pyrolysis Product. Abbreviations: Cocaine (**COC**), Norcocaine (**NCOC**), Norbenzoylecgonine (**NBE**), Cocaethylene (**CE**), Ecgonine ethyl ester (**EEE**), Benzoylecgonine (**BE**), *m*-Hydroxybenzoylecgonine (**MOHBE**), Ecgonine methyl ester (**EME**), Ecgonine (**ECG**), and Anhydroecgonine methyl ester (**AEME**).

On the other hand, the fetus may be able to catalyze the reduction of nitro While potentially able to metabolize xenobiotics like cocaine, this system appears to be saturated with endogenous steroid substrate which would severely limit the metabolism of any additional foreign substrate.[36] groups in drugs as early as 12 weeks of gestation.

This may explain the unexpectedly high incidence of norcocaine found in neonates in a recent study involving a cohort of very low birthweight infants.[16] In experiments involving fetal tissue *in vitro*, this reaction required high levels of coenzymes indicating that the activity of nitroreductase *in vivo* may be slow.[36] Other experiments indicate that enzymes responsible for *N*-demethylation are the most active of the neonatal liver enzymes showing activities that are approximately 30% of adult levels.[37] Moreover, in most experiments involving fetal and neonatal metabolism of drugs, the activity of liver and plasma enzymes are low or nonexistent until birth and then exhibit a steady increase until one year of age.[36,37] In experiments evaluating *o*-demethylation of dextromethorphan, few of the fetal microsomal preparations studied exhibited any *o*-demethylation. The activity in neonatal samples was less than 1% of adult activity and rose to within 25% of adult values within 8 days of birth.[37] The premature neonate may be at special risk to cocaine toxicity because immature hepatic and intestinal enzymes cannot sufficiently deactivate harmful xenobiotics like cocaine.[36] Moreover, these infants will exhibit reduced clearance and

prolonged exposure to cocaine and its metabolites due to incomplete development of glomerular filtration and tubular secretion at birth.[36]

Maternal-Fetal Circulation and Fetal Exposure to Cocaine

Fetal blood is directed through the placenta by way of two umbilical arteries and one vein for the purposes of oxygenation, nutrition, and removal of metabolic wastes. Maternal blood enters the placenta via the uterine and ovarian arteries and diffusion causes oxygen to enter the fetal blood and carbon dioxide to enter the maternal circulation. Nutrients such as glucose and essential amino acids do not cross the placenta by diffusion but instead are transported across the placenta by facilitated and active transport, respectively. Fetal blood is returned via the umbilical vein and then enters the ductus venosus, a large vessel that arises at the junction of the portal and umbilical veins. A small portion of blood is shunted through the liver and then joins with blood from the ductus venosus in the inferior vena cava. This blood passes through the left atrium, left ventricle, and into the aorta, from which it is distributed primarily to the heart muscle, the head, and the upper extremities. The blood is returned to the heart via the superior vena cava where it passes through the right atrium, the right ventricle, and into the pulmonary trunk. The pressure exerted by the unexpanded lungs allows only a small amount of flow to

enter the fetal lungs and from there the blood goes to the viscera and lower extremities. The remainder flows into the descending aorta, and is carried by the paired umbilical arteries to the placenta.

Diffusion in the placenta is not limited to oxygen and carbon dioxide, however, as any small, lipid soluble molecule (endogenous or exogenous) will also readily diffuse across the placenta. Cocaine and its two pharmacologically active metabolites, cocaethylene and norcocaine, fit these criteria and have been found to readily cross the placenta.[38] The surface area and thickness of the placenta contribute in determining the rate of drug diffusion across the membranes. As pregnancy progresses, thinning of the placental membrane and increase in villous surface area facilitate drug passage.[39]

Cocaine is not trapped in the fetal circulation but can diffuse back across the placenta and re-enter the maternal circulation.[38] The same is not true for the more polar metabolites of cocaine. These either diffuse more slowly than cocaine or become trapped on the fetal side where they end up in amniotic fluid or meconium.[40] Experiments with fetal lambs suggest that fetal exposure to cocaine and its metabolites is prolonged because of this slow equilibration and hence may result in increased toxicity for the fetus.[40]

Adverse Effects Associated with Cocaine Use

Much research has been devoted to identifying complications associated with the use of cocaine. Death from respiratory collapse, myocardial infarction and ischemia, subarachnoid hemorrhage, thermoregulatory failure, and cerebrovascular infarction have all been reported.[1,2,7,41] Other, nonlethal, complications include chronic nasal problems, "crack lung", crack keratinitis", vitamin deficiencies, sexual difficulties, psychosis, agitation, delirium, and neurotoxicity.[1,2,7,14,41,42]

In addition to the effects listed above, adverse effects experienced by pregnant women from cocaine use have also been reported. Cocaine affects pregnancy, causing vasoconstriction, uterine contractility, and hypertension through a catecholamine-mediated process. This can result in premature rupture of the membranes which can put the fetus at increased risk for infection. However, there is conflicting evidence for an increase of this problem associated with cocaine use. The incidence of premature rupture of the membranes was increased among cocaine users with positive urine tests when compared to admitted cocaine users with negative urine tests.[43] Conversely, when subjects were matched for degree of prenatal care, there was no increased risk for cocaine using subjects versus control subjects.[44]

The incidence of *abruptio placenta* associated with cocaine use has been extensively studied and it is generally agreed that maternal cocaine users have an increased risk of experiencing this complication.[33] Even in studies controlling for lack of prenatal care, cocaine was found to be an independent risk factor for placental abruption.[44,45] The association between cocaine and placental abruption seems creditable as this complication has long been associated with hypertension, and cocaine use during pregnancy causes significant, dose-dependent increases in maternal blood pressure.[8,46]

The associated hypertension and vasoconstriction that accompanies maternal cocaine use are also responsible for decreased placental blood flow and increased uterine contractility.[9,34] In combination these factors may result in spontaneous abortion, pre-term delivery, and pre-eclampsia-like syndrome, all of which have been reported in cases of maternal cocaine use.[9,34,42] The pre-eclampsia-like syndrome is characterized by acute hypertension, edema and proteinuria, but unlike true pre-eclampsia, the symptoms will usually resolve in 8-12 hours with supportive care.[42] Pulmonary edema from cocaine bronchiolitis is another life threatening condition that maternal cocaine users may also experience. The mortality rate associated with this condition approaches 33% since the only treatment is supportive therapy in an intensive care unit.[42] Additional complications include seizures, cardiac arrhythmias, poor weight gain and nutrition, due to cocaine induced anorexia, and sudden death.[2,7,9,14,42]

Table 1-1. Adverse Effects Associated with Gestational Cocaine Exposure.

Adverse Effect	Reference	Author	Year	Subjects (n)
Cerebral hemorrhage	47	Spires et al.	1989	1
Congenital anomalies	48	Hoyme et al.	1990	10
Intrauterine growth retardation	48	Hoyme et al.	1990	10
Meconium stained amniotic fluid	49	Tabor et al.	1990	37
Myocardial calcification	50	Yap et al.	1994	1
Neurobehavioral abnormalities	51	Lester et al.	1991	22
Renal vascular abnormalities	52	Ho et al.	1994	3
Small head size	53	Chiriboga et al.	1993	14
Subcutaneous fat necrosis	54	Carraccio et al.	1994	1
Sudden infant death syndrome	55	Ward et al.	1986	3
Stillbirth	56	Ryan et al.	1987	4

There are also many reports linking fetal and neonatal difficulties to maternal cocaine use during pregnancy. The difficulties, collated from case reports and small studies, are listed in Table 1-1, along with the appropriate references. Large clinical studies have generally reinforced these case reports by documenting an increase of many of these problems in exposed neonates versus controls. According to these studies, such infants were more likely to remain in the hospital after their mothers were discharged, present with lower apgar scores and poorer general outcomes than matched controls, and to be pre-term, low birth weight, and resuscitated at birth.[9,10,56,57]

Many of these problems can be linked to the hypertension and decreased placental blood flow that result from maternal cocaine use. In animal studies, these effects are accompanied by marked fetal hypoxia, hypertension, and

tachycardia.[34] The most obvious consequence of the decreased placental blood flow is decreased oxygen and nutrient transfer to the fetus which culminates in intrauterine growth retardation. A decrease in mean fetal weight was shown to be independent of maternal weight gain in pregnant rats following administration of cocaine.[58] This work is important because it suggests that cocaine exerts a direct effect on fetal growth and is not simply a function of maternal malnutrition.[33] The same observations are noted in studies involving humans. In a study comprised of 366 infants born to cocaine using women and a set of control infants, the mean birth weight of the cocaine exposed infants was 376 grams less than controls matched for factors associated with low birth weight.[59] Another study in which groups were matched for racial distribution, cigarette use, maternal age, maternal weight gain, and parity, the mean birth weight for cocaine exposed infants was 2829 grams versus 3436 grams for controls. In addition 28% of the cocaine exposed infants were <2500 grams at birth versus 5% of controls.[60]

A smaller than normal neonatal head circumference has also been associated with maternal cocaine use. The microcephaly seen in these infants appears to be the result of an *in utero* cerebral hemorrhage and is the most common brain abnormality observed in infants of cocaine abusing mothers.[60-62] Evidence for this includes postmortem studies of stillborn infants and computerized tomographic images of neonates born to cocaine using women

show a higher incidence of bilateral cerebral hemorrhages.[60,61] Additionally, increased cerebral blood flow velocity, a risk factor for intracranial hemorrhage, has been observed in cocaine exposed neonates.[63]

Congenital anomalies associated with maternal cocaine abuse may also be the result of altered or interrupted blood flow patterns during development. Animal studies support the view that hemorrhage or infarct leads to necrosis and disruption of both developing and fully formed structures. Sprague-Dawley rats treated with cocaine during gestation were noted to have hemorrhages involving the head, limbs, tail, and urogenital track.[64] These are the same patterns of anomalies most commonly seen in humans in association with prenatal cocaine exposure. A study involving 119 cocaine exposed infants and 100 drug-free controls supports the association between prenatal cocaine exposure and congenital anomalies by showing an increased risk of 6.5 for genitourinary abnormalities in the cocaine exposed group.[65] Other studies support this view by showing an increased risk for genitourinary abnormalities[66], congenital heart defects[50], and multiple anomalies.[67,69]

The association between prenatal cocaine exposure and increased risk for congenital abnormalities is controversial. Several retrospective and prospective studies have not been able to demonstrate a significant increase of congenital anomalies among cocaine exposed infants.[57,70,71] These studies (as with many studies showing increases in adverse effects in cocaine exposed

infants) may have been limited by small sample size and/or failure to control for confounding variables such as polydrug use, nutritional and socioeconomic status, and prenatal care. The problem of small sample size (but not other confounders) in a study can be overcome by the use of a meta-analysis technique to analyze data from multiple reports. For example, Lutiger et al. used this technique to analyze reports that appeared up to 1989 and found a significant increase in genitourinary tract malformations in cocaine exposed infants versus controls.[72]

Scope of the Project

This project involves the qualitative and quantitative determination of cocaine and its metabolites in biological specimens collected from women and their neonates during the period 8/94 to 12/95. The purpose of this project was to use the data generated by the analyses of the specimens to examine differences in the ability of the specimen types to reflect maternal cocaine use and identify a definitive measure for determining prenatal cocaine exposure. The need for this is evident when examining the limitations of immunochemical screening, the most common method for determining prenatal cocaine exposure.

Immunochemical screening methods for detection of drug use during pregnancy are usually performed on urine obtained from either the mother or

infant. While urine may be the most convenient specimen for the detection of drugs, reliable detection of cocaine metabolites (primarily benzoylecgonine) in this medium is limited by the relatively short biological half-life of these analytes. False negative test results are a significant problem in methods designed to detect drugs and/or their metabolites in urine since elimination is variable and occurs over a limited period of time following cocaine exposure. In several recent studies, urine immunoassay for benzoylecgonine failed to identify 22-60% of cocaine exposed infants.[16,56,73,74] The subjects participating in this project and the specimens collected from them are outlined below.

Pregnant subjects who were admitted to the labor and delivery service at Shands Hospital at the University of Florida (Gainesville, FL) were recruited to participate in this study. Targets and controls were identified by chart review. Subjects admitting to cocaine use during the current pregnancy were asked to participate in this study as a target. Subjects denying cocaine use were recruited to participate as controls. All maternal subjects were ≥18 years of age. Participants were interviewed confidentially to assess the amount and timing of drug use throughout the pregnancy.

Selection and exclusion from a pool of potential subjects was performed by a physician investigator who was blinded to drug history. Excluded from the study were subjects admitting to consuming alcohol at >40 grams of absolute alcohol per day, or using the following drugs chronically: azathioprine,

amphetamines, benzodiazepines, barbiturates, cyclosporine, opiates, methadone, methaqualone, or steroids. Also, excluded was any woman with a chronic disease known to affect the immune system or condition that might affect mental status such as mental illness or retardation. The study protocol was approved by the University of Florida Institutional Review Board, and informed consent was obtained from all subjects.

The following matrices were collected, when possible, from the mothers and their neonates: maternal hair, amniotic fluid, neonatal urine, meconium, umbilical cord tissue, and colostrum. The matrices were analyzed for the following cocaine analytes: cocaine, ecgonine methyl ester, benzoylecgonine, cocaethylene, norcocaine, ecgonine ethyl ester, benzoylnorecgonine, and *m*-hydroxybenzoylecgonine. Maternal hair and colostrum were analyzed by gas chromatography-mass spectrometry (GC/MS). All other matrices were analyzed by both GC/MS and high performance liquid chromatography (HPLC). In addition, neonatal urine and meconium were screened for the cocaine metabolite, benzoylecgonine by enzyme multiplied immunoassay (EMIT), and cloned enzyme donor immunoassay (CEDIA). The total number of subjects, number of specimens collected, and a breakdown of targets and controls by specimen type are presented in Table 1-2.

Table 1-2. Numbers of Subjects Enrolled and Specimens Collected in Each Category by Specimen Type.

Subjects	Enrollment Status (n)	Hair	Urine	Meconium	Cord Tissue	Colostrum	Amniotic Fluid
Targets	34	26	31	32	28	10	13
Controls	49	40	44	42	42	11	19
Total	83	66	75	74	70	21	32

Scope of Dissertation

This dissertation presents the analytical results from the detection of cocaine and its metabolites in a variety of biological specimens of maternal and neonatal origin in order to examine differences in the abilities of the specimen types to reflect maternal cocaine use. Chapter 1 presented an introduction to the history of cocaine use, the pharmacology and metabolism of cocaine, mechanisms by which the unborn fetus is exposed to cocaine and its metabolites, and the adverse effects associated with the abuse of cocaine.

Chapter 2 discusses the detection of cocaine and its metabolites in maternal hair and the use of hair for determining prenatal cocaine exposure in the current study. In addition, a review of the literature concerning the detection of cocaine and its metabolites in hair and the current view on how cocaine and metabolites are incorporated into hair are presented.

Chapter 3 introduces the detection of cocaine and its metabolites in three rarely used specimens, amniotic fluid, colostrum and umbilical cord tissue. A

review of the literature and a discussion of how these specimens might contribute to the detection of prenatal cocaine exposure are included.

Chapter 4 discusses the detection of prenatal cocaine exposure by analysis of cocaine and its metabolites in the most commonly used specimens neonatal urine and meconium. Specifically, the following questions regarding the use of meconium for detecting prenatal cocaine exposure will be addressed: (1) Does meconium provide more sensitivity for the detection of prenatal cocaine exposure? (2) Are the concentrations of cocaine analytes higher in meconium when compared to urine? (3) Does meconium provide a longer window for the detection of prenatal cocaine exposure? (4) Does the cocaine metabolite *m*-hydroxybenzoylecgonine provide greater sensitivity for the detection of prenatal cocaine exposure when using meconium?

Chapter 5 compares the sensitivities among the different specimens analyzed in this project. In addition, the overall results of the project are reviewed to examine which specimen or combination of specimens is best for the detection of prenatal cocaine exposure. Chapter 6, the final chapter, summarizes overall conclusions and presents ideas for future work.

CHAPTER 2
UTILITY OF MATERNAL HAIR FOR DETERMINING PRENATAL COCAINE EXPOSURE

Introduction

The development of more sensitive methods for determining fetal drug exposure is the goal of many scientists seeking to answer questions regarding drug exposure and fetal outcome. The analysis of maternal hair for drugs of abuse has the potential for providing a record of drug use dating back to the beginning of the gestational period. This is because hair grows at a relatively constant rate and drugs are easily incorporated into hair. The purpose of this work was to assess the ability of maternal hair to produce a record of the degree and timing of maternal cocaine use and examine the sensitivity of maternal hair for detecting prenatal cocaine exposure.

Hairs are filamentous appendages of the epidermis. Each hair has a shaft and a root and is comprised of modified keratin. Within the dermis, the hair is surrounded by the cells of the hair follicle, a funnel-shaped invagination of the skin that supports the hair root. The root, with its densely packed melanocytes, is referred to as the hair bulb and is deeply indented to

accommodate capillaries that act to nourish the follicle and regulate body temperature. This indentation is termed the dermal papilla. The ducts of one or more sebaceous glands open into each follicle. Sweat glands are also closely associated with the hair structures. Figure 2-1 depicts the hair and its structures, as described in this paragraph.

The growth of hair can vary with anatomical site, thickness, race, and sex. Growth averages from a low of 0.21 mm/day for eyebrow hair to a high of 0.44 mm/day for scalp hair. The rate of hair growth also varies for different regions of the scalp; however the average is 0.35 mm/day, or approximately 1 cm/month.[75] Additionally, there are three phases of hair growth, anagen, catagen, and telogen. Fast growing hairs are said to be in the anagen phase, which can last up to 10 years in the large hairs present on the scalp. Eighty to 90% of all hairs are actively growing and are said to be in the anagen phase. Approximately 1% of all hairs are in the catagen phase, a transition phase that lasts several weeks during which the hair growth slows then stops. The remaining 10-20% are in the telogen phase, which lasts for several months. It is during this phase that the hair is pushed out by a new hair growing in its place. Hair in the telogen phase is also easily pulled out of the epidermis, as it is no longer anchored by the dermal papilla.[75]

Unlike some animals in which hair growth and shedding are seasonally synchronized, the cycles of growth and hair loss in humans occur randomly, so

that hair loss is not an overtly noticeable event. This is not to say that human hair is not affected, at least minimally, by hormonal fluctuations. Hypothyroid individuals have been found to have a higher percentage of telogen hairs, and when treated with exogenous thyroid hormone, the percentage of hair in the telogen phase returns to normal.[76] Higher levels of ovarian and adrenocortical steroid hormones that normally occur during pregnancy may also affect the numbers of actively growing hairs. Studies show that the percentage of hairs in the actively growing anagen phase increases as pregnancy progresses, peaking at 98% by the ninth month.[76] This may be due to a suspension of normal and continuous shedding during the third trimester. The result is the well known occurrence of postpartum alopecia, which is typified by a significant loss of hair, approximately 2-3 months after parturition.[76]

Analysis of hair for exposure to toxic metals such, as arsenic, lead, cadmium, and mercury, have been performed routinely since the 1950s.[77] The realization that hair might serve as a historical record of drug use occurred in 1954, when researchers reported that hair from guinea pigs contained high concentrations of phenobarbital after habitual dosing of the drug.[77] The method used to detect the barbiturate, required large sample sizes and did not provide sufficient sensitivity for more than a passing interest in this area.[77]

In the 1970s, development of sensitive and specific methods of drug detection and identification, such as radioimmunoassay (RIA) and GC/MS,

respectively, brought with it a renewed interest in detecting drugs in hair. In 1979, the detection of drugs in human hair was first reported. The researchers used RIA to detect opiates in the hair of heroin addicts and demonstrated that concentration differences between the scalp and the distal end correlated with the length of time the drugs were used.[78] During the 1980s, more researchers began to analyze hair for drugs, and by the end of the decade, the detection of cocaine, nicotine, phencyclidine, methamphetamine, and other drugs of abuse had been reported.[77]

At the same time, other researchers were searching for a satisfactory explanation for how drugs are sequestered in hair. Initially, it was thought that drugs entered the hair shaft exclusively by diffusion from the capillary beds that supplied the hair and its follicle.[79] But the data generated from the analysis of cocaine in hair could not be adequately explained by this model. These scientists found that cocaine concentrations were greater than benzoylecgonine concentrations in hair.[79] This is the opposite of what is expected if blood plasma is the sole source for cocaine in hair. An alternative model was subsequently developed which proposes that ingested drugs can be deposited in hair by several mechanisms including (a) diffusion from capillary beds that supply the hair bulb and follicle; (b) the wetting that occurs when drug laden sweat bathes the hair, and; (c) wetting of the hair shaft by drugs contained in sebum, which is deposited onto the developing hair late in the growth process.[79]

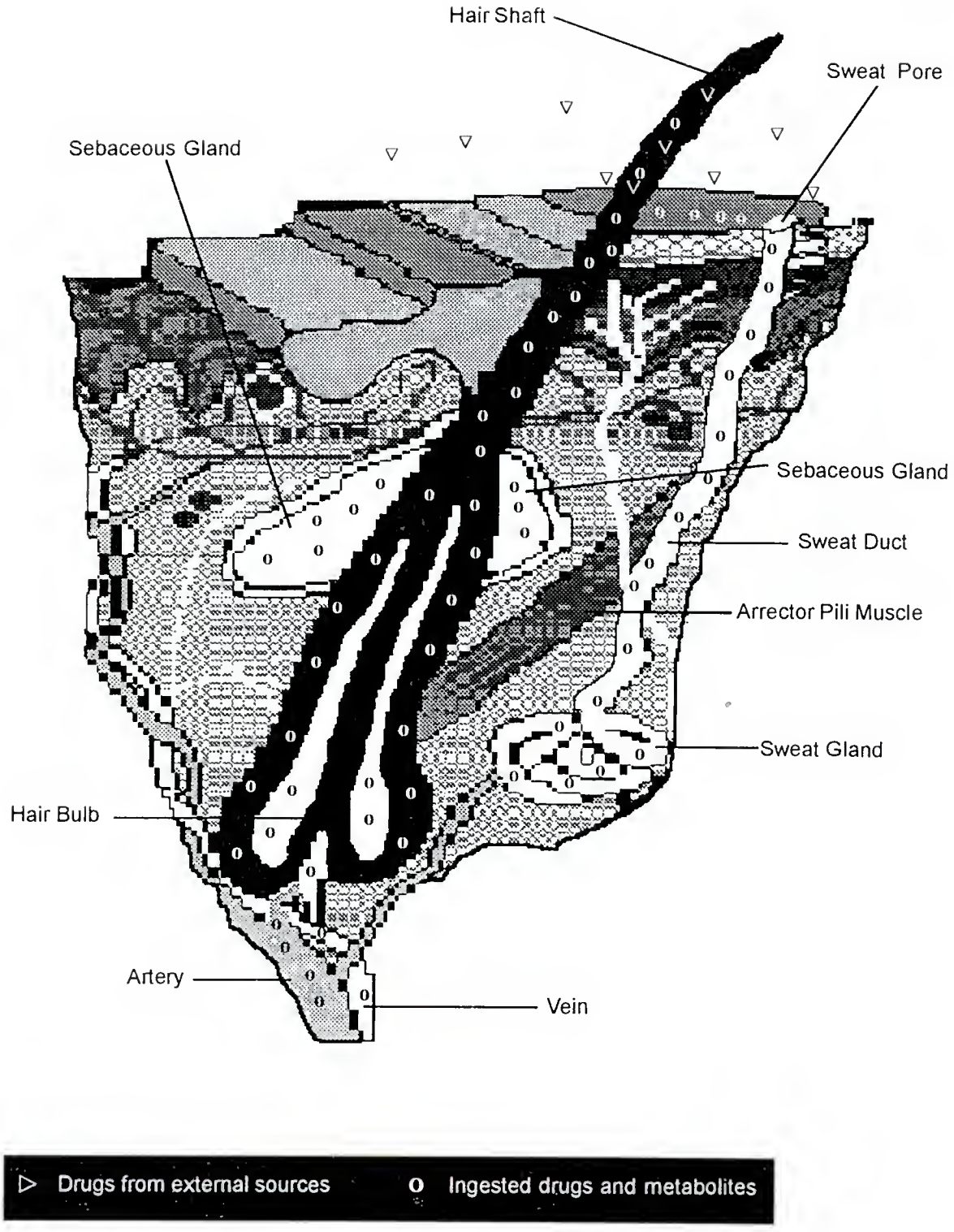


Figure 2-1. Current view on the mechanisms by which drugs are thought to enter hair.

Evidence for this model includes analysis of sweat for cocaine, in which cocaine was the analyte detected most often and in the highest concentrations, followed by ecgonine methyl ester and benzoylecgonine.[80] Further evidence is provided by an experiment in which five volunteers were administered deuterated codeine and then asked to hold drug free hair in their hands for 30 minutes. Deuterated codeine was detected in all specimens, presumably due to the wetting of the hair by the drug laden sweat excreted from the palms of the volunteers.[79] This model also allows for the possibility that externally applied drugs, whether in solid or vapor phase, can enter the hair when it is wetted during normal hygiene Figure 2-1 illustrates the different mechanisms by which drugs are thought to enter hair.

The majority of scientific studies investigating human hair analysis for detecting drug use have utilized the following populations: (a) highly controlled clinical studies with small numbers of volunteers, (b) coca-leaf chewers, (c) individual case reports, and (d) prison or county jail detainees.[81] The use of neonatal hair to detect prenatal cocaine exposure has been reported less often. Graham et al. reported the results of RIA analysis of hair for the cocaine metabolite, benzoylecgonine, from 7 neonates whose mothers admitted to cocaine use during their pregnancy. Hair from all 7 neonates was reported positive for benzoylecgonine.[82] A similar study by Klein et al. reported a method for analyzing neonatal hair for cocaine and nicotine by RIA. Hair from 6

neonates whose mothers admitted using both cocaine and nicotine during their pregnancy was found to be positive for both analytes.[83] In one of two large studies reported to date, Callahan et al. utilized the services of a commercial laboratory to analyze the hair from 53 neonates for cocaine by RIA. In addition, 59 maternal hair samples were collected and analyzed. Forty of the maternal samples and 25 of the neonate specimens tested positive for cocaine.[73]

The use of neonatal hair to detect prenatal cocaine exposure has significant disadvantages when compared to maternal hair. These include (a) limited amounts of material; (b) mothers are often unwilling to part with the small amounts of hair present on their new infant's head, and, most importantly, (c) neonatal hair does not develop until the last trimester of pregnancy and therefore is only representative of the last 12 weeks of gestation. Conversely, maternal hair is usually present in ample amounts and, if of sufficient length, is representative of the entire gestational period. Despite these advantages, only five studies utilizing maternal hair to detect gestational cocaine use have been reported. In two of these, Callahan et al. and Grant et al. reported the use of RIA to detect cocaine in 59 and 148 maternal hair samples, respectively.[73,84] In the third study, Marques et al. used RIA to detect cocaine in the hair of 63 mother-infant pairs.[85] Finally, DiGregorio et al. used the more specific method of GC/MS in two studies to test for cocaine, benzoylecgonine, and cocaethylene in

hair from randomly selected women who presented for delivery without prenatal care.[86,87]

This chapter reports a GC/MS method for determining cocaine and the cocaine metabolites; benzoylecgonine, ecgonine methyl ester, cocaethylene, norcocaine, *m*-hydroxybenzoylecgonine, and ecgonine ethyl ester in maternal hair.

Materials and Methods

Subjects

Subjects admitting to cocaine use during their current pregnancy were asked to participate in this study as a target (n=34). Women admitted to labor and delivery that were not known to be cocaine users were recruited to participate as controls (n=49). Participants were interviewed confidentially to assess the amount and timing of drug use during each trimester.

Specimens

Hair specimens were collected during a postpartum interview. The hair was cut as close to the scalp as possible in the vertex region of the head and approximately 25-50 strands were taken. The hair was attached to an index card with cellophane tape and the root end was identified on the card, along with the subject's identification number. The hair and card were wrapped in aluminum

foil and the hair was stored at room temperature until analysis. All hair specimens were marked with a unique research identification number and the analyst was blinded to drug history.

Chemicals

Benzoylecgonine (BE), cocaine (COC), ecgonine methyl ester (EME), cocaethylene (CE), norcocaine (NCOC), and trideuterated analogs of BE, EME, COC, and CE were purchased from Radian Corporation (Austin, TX). Ecgonine ethyl ester (EEE) and *m*-hydroxybenzoylecgonine (MOHBE) were a generous gift from Edward Cone, Ph.D. Methyl-(tert-butyldimethylsilyl)-trifluoracetamide (MTBSTFA) was purchased from Pierce Chemical Company (Rockford, IL). All other reagents were of reagent grade and were purchased from Fisher Scientific (Orlando, FL).

Standard Preparation

Calibration standards were prepared by fortifying 10 mg of blank hair with aliquots of standard solutions to form a calibration curve in the range of 1-50 ng/mg. Control specimens at 0, 7.5, and 20 ng/mg, for all analytes were included in each analytical run. In addition, a 50 ng/mg cocaine standard was included in each run to monitor hydrolysis of COC to BE or EME during

extraction and subsequent analysis. All calibration and control standards were prepared immediately prior to analysis.

Specimen Preparation and Wash Procedure

Specimens were cut into 3 cm lengths starting at the root end and moving distally, to represent the third, second, and first trimesters, respectively. Any lengths longer than 9 cm were discarded. Specimens shorter than 9 cm were labeled according to the trimesters represented.

The sectioned hair was placed in a labeled glass culture tube and was washed with 3 mL of methanol by vortexing briefly to remove externally applied drugs, dirt and oils from the exterior of the hair. The methanol wash was decanted into a labeled culture tube and extracted and analyzed for cocaine and metabolites as described below. The washed hair was transferred to a clean, labeled culture tube and dried at 50°C.

Once dry, the hair was transferred to a labeled 2.0 mL plastic screw-cap microtube (#10832, Biospec Products, Bartlesville, OK) and was pulverized to a powder in a Mini-Beadbeater-8™ cell disrupter (Biospec Products, Bartlesville, OK). Ten to 20 milligrams of hair powder was weighed into a labeled culture tube for extraction and analysis.

Extraction

The solid phase extraction (SPE) of cocaine and its metabolites and subsequent analysis by GC/MS was adapted from procedures previously described by Cone et al. and Goldberger et al.[27,88]

Twenty-five microliters of deuterated internal standard solution containing 4 ng/μL each of trideuterated analogs of BE, EME, COC, and CE was added to each specimen. Next, 3 mL of methanol was added to each specimen, the specimen tubes were capped and placed into heating blocks to reflux at 40°C for 18 hours. After refluxing, specimens were centrifuged for 10 min. at 1000 x g and the methanolic extracts were decanted into labeled culture tubes and dried under a gentle nitrogen stream.

The dried extracts were reconstituted in 3 mL of phosphate buffer (0.025M, pH 4) and applied to CleanScreen™ SPE extraction columns (United Chemical Technologies, Horsham, PA) that had previously been conditioned with elution solvent (1 x 1 mL), methanol (1 x 3 mL), deionized water (1 x 3 mL), and 0.025M (pH 4) phosphate buffer (1 x 2 mL). The specimens were followed with a wash of deionized water (1 x 2 mL) and 0.1M HCl (1 x 2 mL). The columns were then air dried under full vacuum for 2 min. A methanol wash (1 x 6 mL) and a second 2 min drying step completed the washes. The analytes were then collected in culture tubes by eluting with (1 x 8 mL) of the elution solvent (methylene chloride: isopropanol: concentrated aqueous ammonium

hydroxide, 80:20:2 by vol). The extracts were evaporated to dryness at 40°C under a gentle nitrogen stream. The extracts were derivatized by adding MTBSTFA (30 μL) and heating at 90°C for 60 min.

Gas Chromatography/ Mass Spectrometry (GC/MS)

GC/MS analyses were performed with a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a Hewlett-Packard 7673A automated liquid sampler and interfaced with a Hewlett-Packard 5972A mass selective detector. The GC and detector were controlled by a Hewlett-Packard data system. The GC column was a HP-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness). Helium was the carrier gas, programmed at a constant flow rate of 1.0 mL/min. The injection port was fitted with a 2-mm silanized glass liner. The injection port temperature was 275°C, and the detector temperature was 290°C.

The initial oven temperature of 90°C was maintained for 1 min, programmed to 220°C at 30°C /min and held for 0.5 min, then programmed to 330°C at 20°C /min and maintained for 1 min. The total run time was 12.33 min. The GC/MS was operated in the selected ion monitoring (SIM) mode with a dwell time of 20 ms/ion and quantitation was based upon ion peak area ratios of analyte to internal standard. The quantitation and identification ions for each analyte and internal standard are listed in Table 2-1.

Results

The calibration curves for each analyte were found to be linear over the calibration range by use of the standard error of the estimate statistic (Appendix I, Curves I-1 to I-7). Limit of detection (LOD) was defined as the concentration corresponding to a signal to noise ratio of 3. The limit of quantitation (LOQ) was determined by the analysis of a series of decreasing standards and defined as the lowest standard that did not deviate from the target concentration by more than 20%. Recovery and precision were measured at two concentrations, level one at 7.5 ng/mg and level two at 20 ng/mg. The LOD, LOQ, recovery, and precision are presented in Table 2-2.

Figure 2-2,A-D illustrates a SIM chromatogram of an unextracted standard (A), a 10 ng/mg extracted standard (B), a negative quality control specimen (C) and a positive hair specimen obtained from a target subject (D). The target specimen was found to contain cocaine (27.6 ng/mg), benzoylecgonine (2.1 ng/mg) and cocaethylene (0.4 ng/mg).

Sixty-six out of 83 subjects (80%) agreed to provide hair specimens for the study. Among targets and controls, 40 out of 49 controls (82%) and 26 out of 34 targets (76%) provided specimens for analysis. Fifty-four out of the 66

specimens (82%) were 9 cm or longer. Numbers of hair specimens by trimester, subject, self report, and result are presented in Table 2-3.

Cocaine, cocaine metabolite, and specimen wash results for each positive specimen are presented in Table 2-4. Cocaine was present in 18% of all specimens tested and 69% of positive specimens, with an average concentration of 9.79 ng/mg. Benzoylecgonine was the most common analyte detected, present in 25% of all specimens and 100% of positive specimens, with an average concentration of 1.72 ng/mg. Trace amounts of cocaethylene, ecgonine methyl ester, and norcocaine were present in 7, 23, and 5% of positive specimens, respectively. Thirteen percent of all wash specimens were positive for one or more cocaine analytes.

Overall agreement between subject's drug use history and GC/MS results was 83% and is shown in Table 2-5. Agreement by trimester is represented graphically in Figure 2-3. In general, agreement among controls was significantly higher than targets ($p < 0.005$, Yates corrected chi-square) and target agreement was highest in the specimens representing the third trimester and lowest in the first.[89] Statistical analysis (McNemars Test, Normal theory method) did not reveal a significant difference between drug use history of all subjects and GC/MS results.[89] There was not a correlation between the amount of drugs used, as estimated in maternal interview, and total amount of analytes determined in hair. This lack of correlation is illustrated in Figure 2-4.

Table 2-1: Target ions used for quantitation and qualifier ions used for identification of cocaine analytes and internal standards

Analyte	Target Ion (m/z)	Qualifier Ions (m/z)
Ecgonine methyl ester	256	182, 282
Ecgonine ethyl ester	270	196, 282
Norcocaine	168	289
Cocaine	182	272, 303
Cocaethylene	196	272, 317
Benzoylecgonine	346	282, 403
m-Hydroxybenzoylecgonine	282	533, 82
d3-Ecgonine methyl ester	185	259, 285
d3-Cocaine	306	275
d3-Cocaethylene	199	275, 320
d3-Benzoylecgonine	349	285, 406

Table 2-2. Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery, and Precision of Cocaine Analytes in Maternal Hair.

Analyte	LOD*	LOQ*	Recovery (%)		Within Run Precision (%)		Between Run Precision (%)	
			Level 1**	Level 2**	Level 1	Level 2	Level 1	Level 2
EME	0.25	0.5	92	105	2.3	3.5	6.2	6.0
EEE	0.5	1.0	75	56	6.0	3.0	8.3	10.8
NCOC	0.5	1.0	84	88	3.7	2.1	12.1	10.6
COC	0.25	0.5	93	105	1.0	2.2	5.0	5.7
CE	0.25	0.5	88	107	1.4	1.3	3.3	6.7
BE	0.125	0.5	97	104	1.8	2.3	9.2	7.5
MOHBE	0.5	1.0	86	107	7.3	10.3	10.9	10.7

*Units are ng/mg.
** Level 1 is 7.5 ng/mg and Level 2 is 20 ng/mg
Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 2-3. Number of Hair Specimens by Trimester, Subject, Self Report, and Result.

Subject	n	%	Cocaine History (n)	GC/MS Positive (n)	% Positive
Target					
1st Trimester	19	73	18	10	56
2nd Trimester	25	96	16	14	88
3rd Trimester	26	100	17	15	88
Control					
1st Trimester	35	88	0	1	3
2nd Trimester	36	90	0	2	6
3rd Trimester	40	100	0	4	10

Table 2-4. Enrollment Status, Hair Wash Results, and Analytes Detected in Hair.

ID	Enrollment Status	COC*	BE*	CE*	EME*	NCOC*	Wash	Comments
04	T		1.2					
05	T		1.9				+	
08A**	T						+	
08B		TR	1.7				+	
08C		0.7	1.8				+	
10B-C	T	3.4	2.4					
16B	C		1.6					all other specimens negative
16C			1.4					
17A	T		1.5				+	
17B		TR	1.9				+	
17C		0.6	2				+	
18A	C						+	+ infant urine
23B	T	TR	TR					
30A	T		1.1		TR			
30B			0.8					
32	T		0.6					
34C	T	2.3	0.8			TR	+	
41A	T	100	19		TR	TR	+	
41B		37	7		TR		+	
41C		30	6		TR		+	
42A-B	T	5	1.3				+	
42B-C		3	2.1				+	
52C	T		TR					
53A	T	0.5	TR					
53B		0.9	TR					
53C		TR	TR					
56B-C	T	27.6	2.1	0.4				
60A	T	10.9	0.7					
60B		5.6	0.5					
60C		5.6	0.8					
65A	T	TR	TR		TR		+	
65B		TR	TR				+	
65C		TR	TR	TR	TR		+	
68C	T	17.6	2.5		TR		+	
71C	C	TR	TR					+ infant urine
72A	C		TR		TR			all other specimens negative
72B			TR					
73C	C	TR						+ meconium & amniotic fluid
75C	C		TR					+ infant urine
76C	T	11	1	0.5	TR		+	
85C	T	TR	TR					

* Units are ng/mg. **A, B, and C, represent 1st, 2nd, & 3rd trimesters respectively.
Abbreviations: T=target, C=control, COC=cocaine, BE=benzoylecgonine, EME=ecgonine methyl ester, NCOC=norcocaine, and CE=cocaethylene, TR=trace, + =positive.

Table 2-5. Agreement Between Drug Use History and GC/MS Results.

		Drug Use History		
		Positive	Negative	Total
GC/MS Results	Positive	29	**10***	39
	Negative	**16**	97	113
	Total	45	107	152

*Note: Discordant results are presented in boldface.

Table 2-VI. Summary of Maternal Hair Data from Previously Published Studies and the Current Study

Investigator	Self Report	(n)	% Positive	% Negative	Method
Callahan et al., 1992					
	Positive	41	97.5	2.5	RIA
	Negative	18	11	89	
Marques et al., 1993					
	Positive	63	98	2	RIA
	Negative	NA	NA	NA	
DiGregorio et al., 1993					
	Positive	13	100	0	GC/MS
	Negative	5	40	60	
DiGregorio et al., 1994					
	Positive*	40	70	30	GC/MS
	Negative*	NA	NA	NA	
Grant et al., 1994					
	Positive	148	87	13	RIA
	Negative	257	14	86	
Current Study					
	Positive	26	73	27	GC/MS
	Negative	40	12.5	87.5	

*Maternal History not included in study
Abbreviations are as follows: RIA= radioimmunoassay, GC/MS= gas chromatography/mass spectrometry, NA= not applicable.

Figure 2-2A. Total ion chromatograms from an Unextracted Standard. Internal Standards: Trideuterated Ecgonine methyl ester (EME.d3), Trideuterated Cocaine (COC.d3), Trideuterated Cocaethylene (CE.d3) and Trideuterated Benzoylecgonine (BE.d3) Analytes: Ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), *m*-hydroxybenzoylecgonine (MOHBE) are readily detected in the standard.

File : C:\HPCHEM\1\DATA\WINECKER\12SEP95B.03P\002SP001.D
Operator : R. WINECKER
Acquired : 12 Sep 95 4:17 pm using AcqMethod COCMB2.M
Instrument : 5972 - MS
Sample Name: UNEXTRACTED STANDARD
Misc Info :
Vial Number: 1

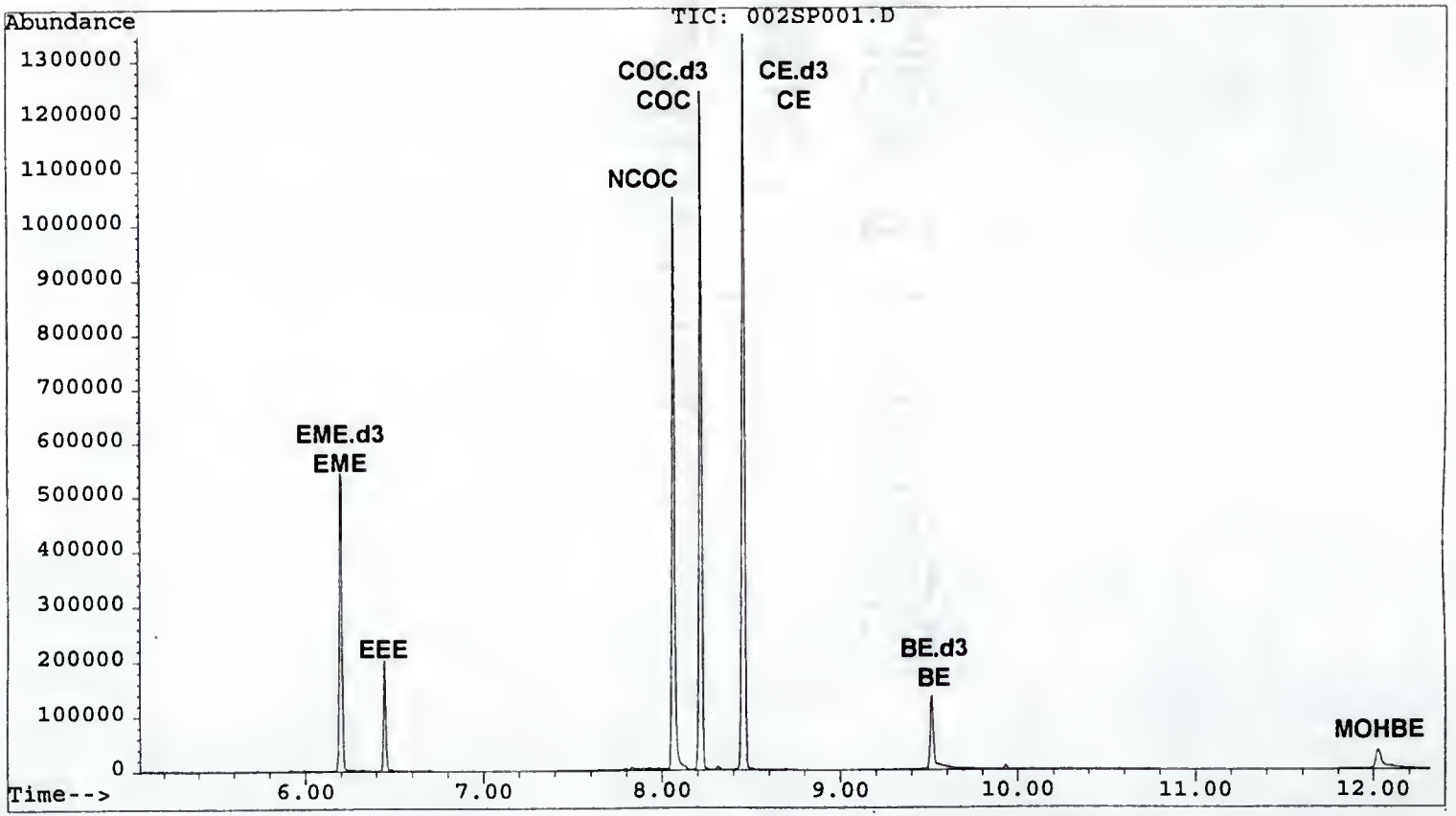


Figure 2-2B. Total ion chromatograms of extracts from a 10 ng/mg extracted standard. Internal Standards: Trideuterated Ecgonine methyl ester (EME.d3), Trideuterated Cocaine (COC.d3), Trideuterated Cocaethylene (CE.d3) and Trideuterated Benzoylecgonine (BE.d3) Analytes: Ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), *m*-hydroxybenzoylecgonine (MOHBE) are readily detected in the standard.

File : C:\HPCHEM\1\DATA\WINECKER\12SEP95B.03P\005SP004.D
Operator : R. WINECKER
Acquired : 12 Sep 95 5:10 pm using AcqMethod COCMB2.M
Instrument : 5972 - MS
Sample Name: 100 NG/10 MG
Misc Info :
Vial Number: 4

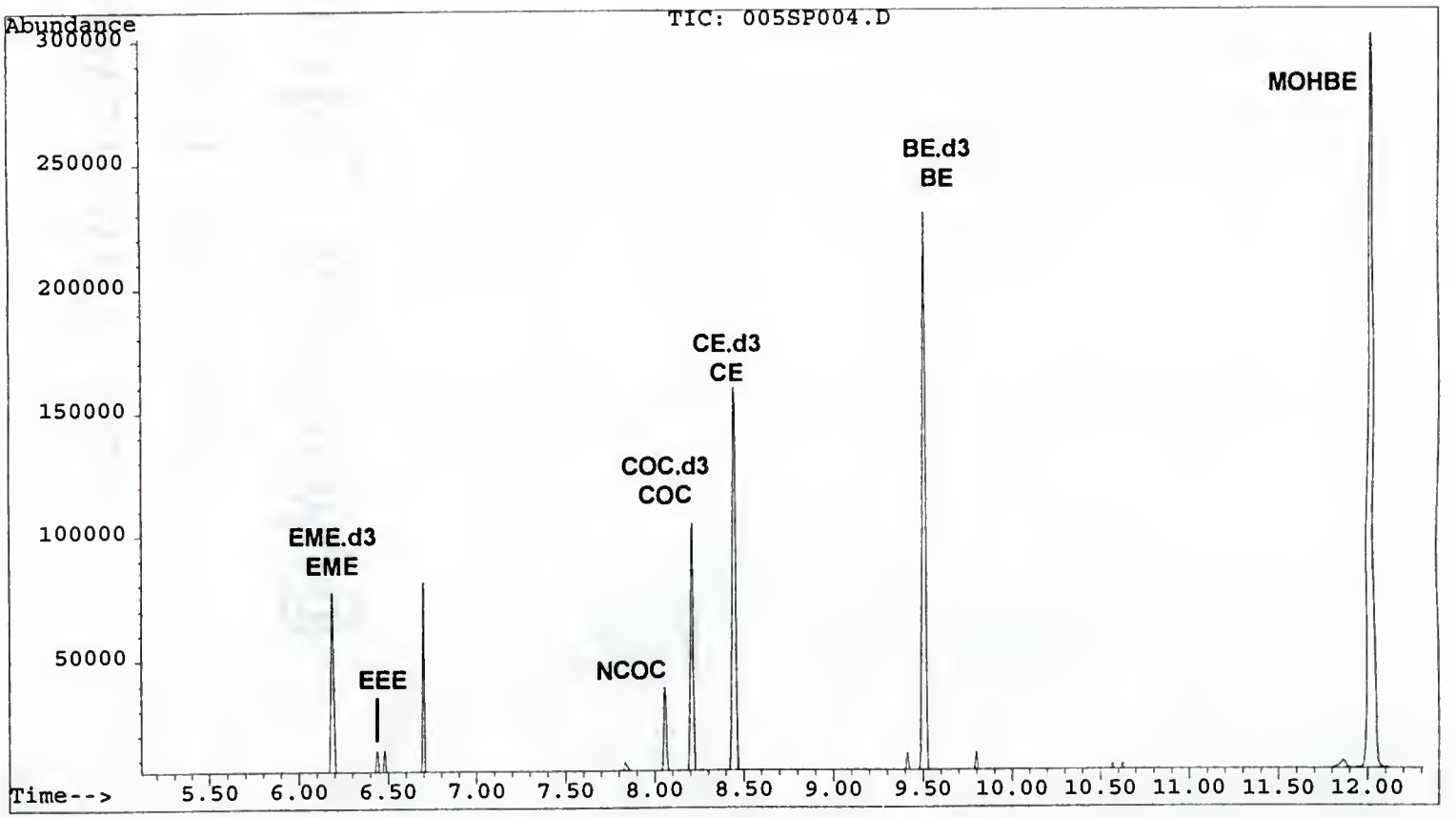


Figure 2-2C. Total ion chromatograms of extracts from a negative quality control standard. Internal Standards: Trideuterated Ecgonine methyl ester (EME.d3), Trideuterated Cocaine (COC.d3), Trideuterated Cocaethylene (CE.d3) and Trideuterated Benzoylecgonine (BE.d3)

File : C:\HPCHEM\1\DATA\WINECKER\12SEP95A.07P\067SP064.D
Operator : R. WINECKER
Acquired : 13 Sep 95 3:08 pm using AcqMethod COCMB2.M
Instrument : 5972 - MS
Sample Name: NEG
Misc Info :
Vial Number: 64

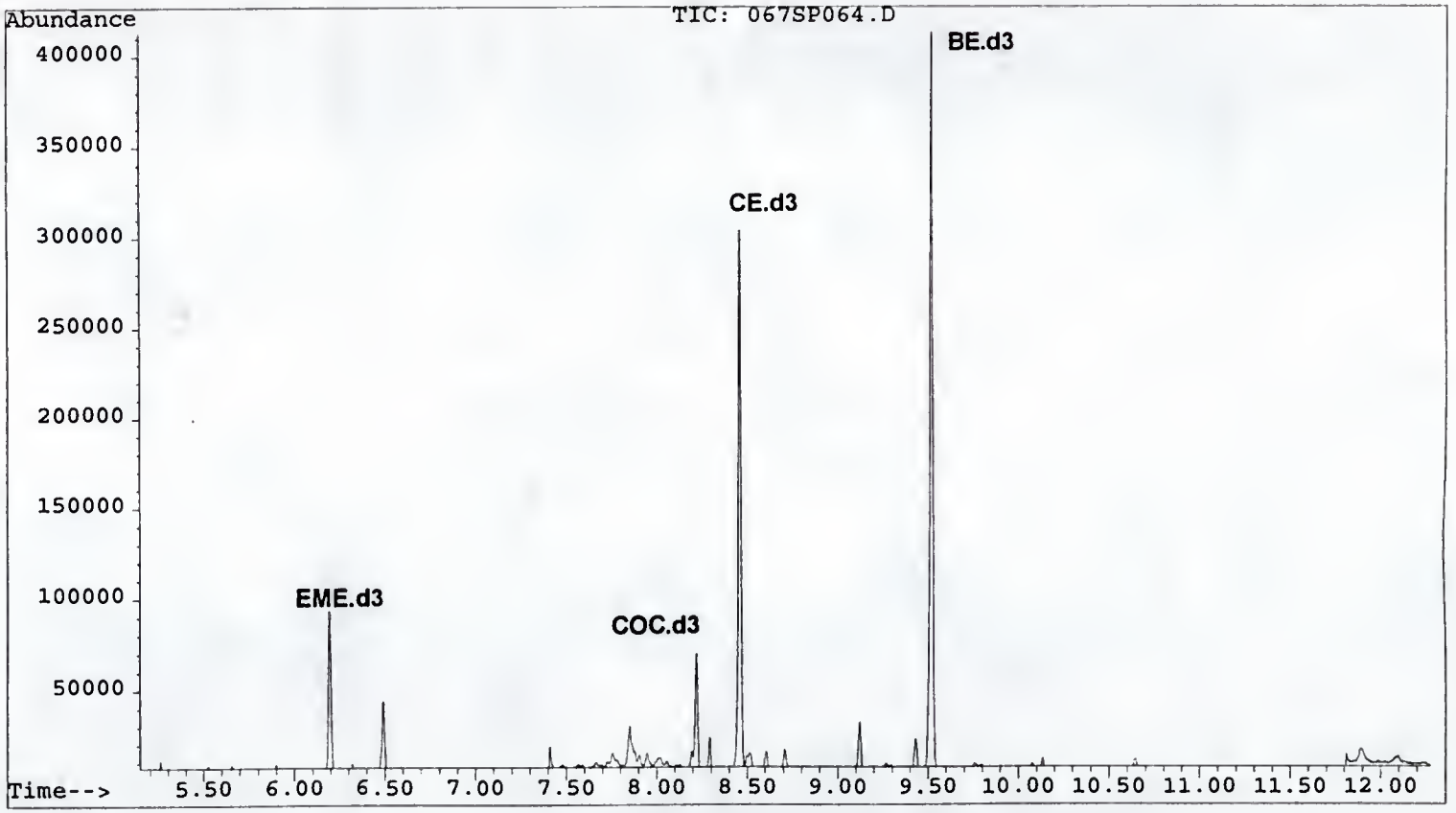
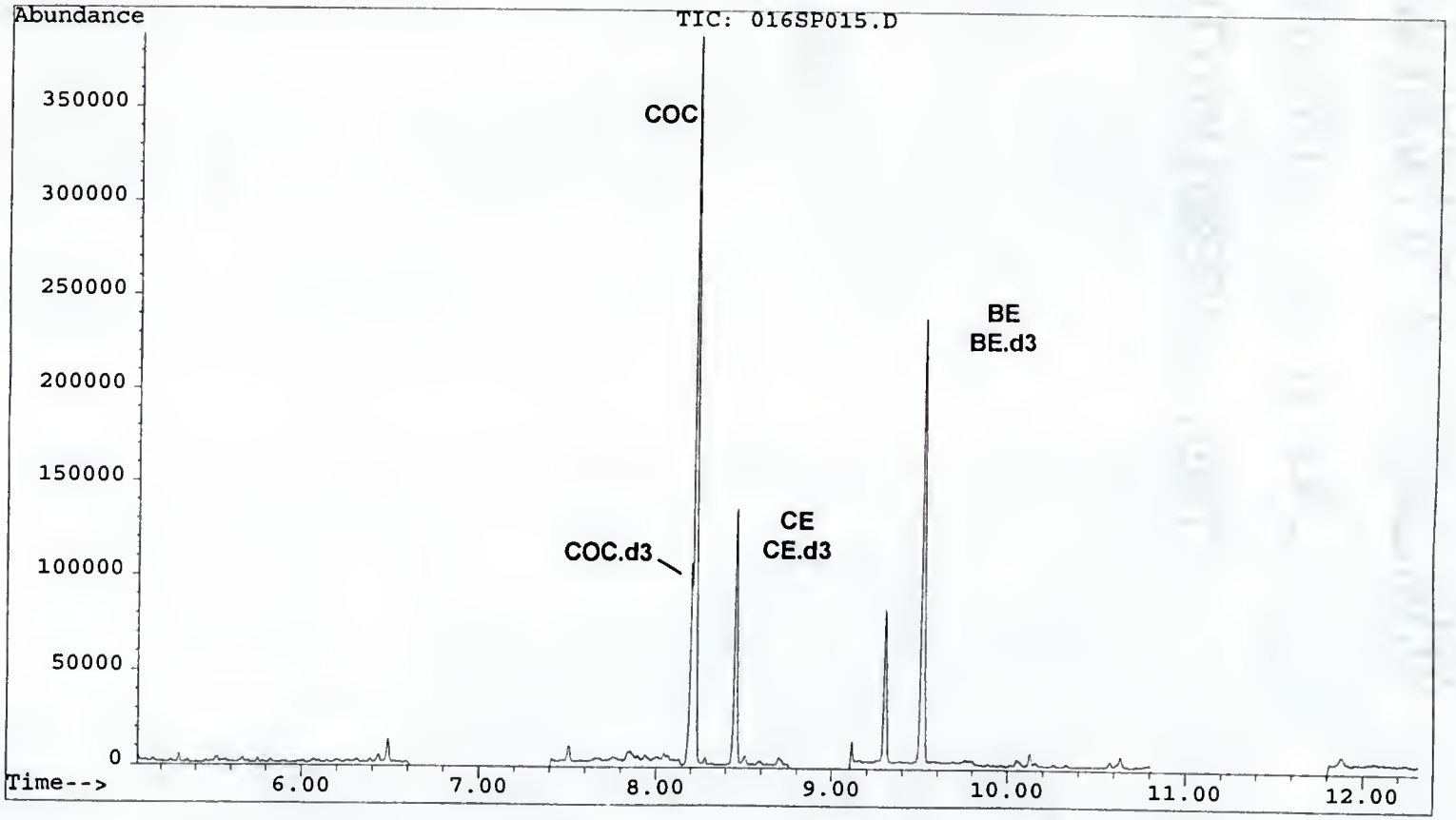


Figure 2-2D. Total ion chromatograms of extracts from a positive hair specimen obtained from a target subject. Internal Standards: Trideuterated Ecgonine methyl ester (EME.d3), Trideuterated Cocaine (COC.d3), Trideuterated Cocaethylene (CE.d3) and Trideuterated Benzoylecgonine (BE.d3) Analytes: The specimen was found to contain 27.6 ng/mg cocaine (COC), 0.4 ng/mg cocaethylene (CE), and 2.1 ng/mg benzoylecgonine (BE).

File : C:\HPCHEM\1\DATA\WINECKER\12SEP95A.07P\016SP015.D
Operator : R. WINECKER
Acquired : 13 Sep 95 12:05 am using AcqMethod COCMB2.M
Instrument : 5972 - MS
Sample Name: CS1056; 16.2MG
Misc Info :
Vial Number: 15



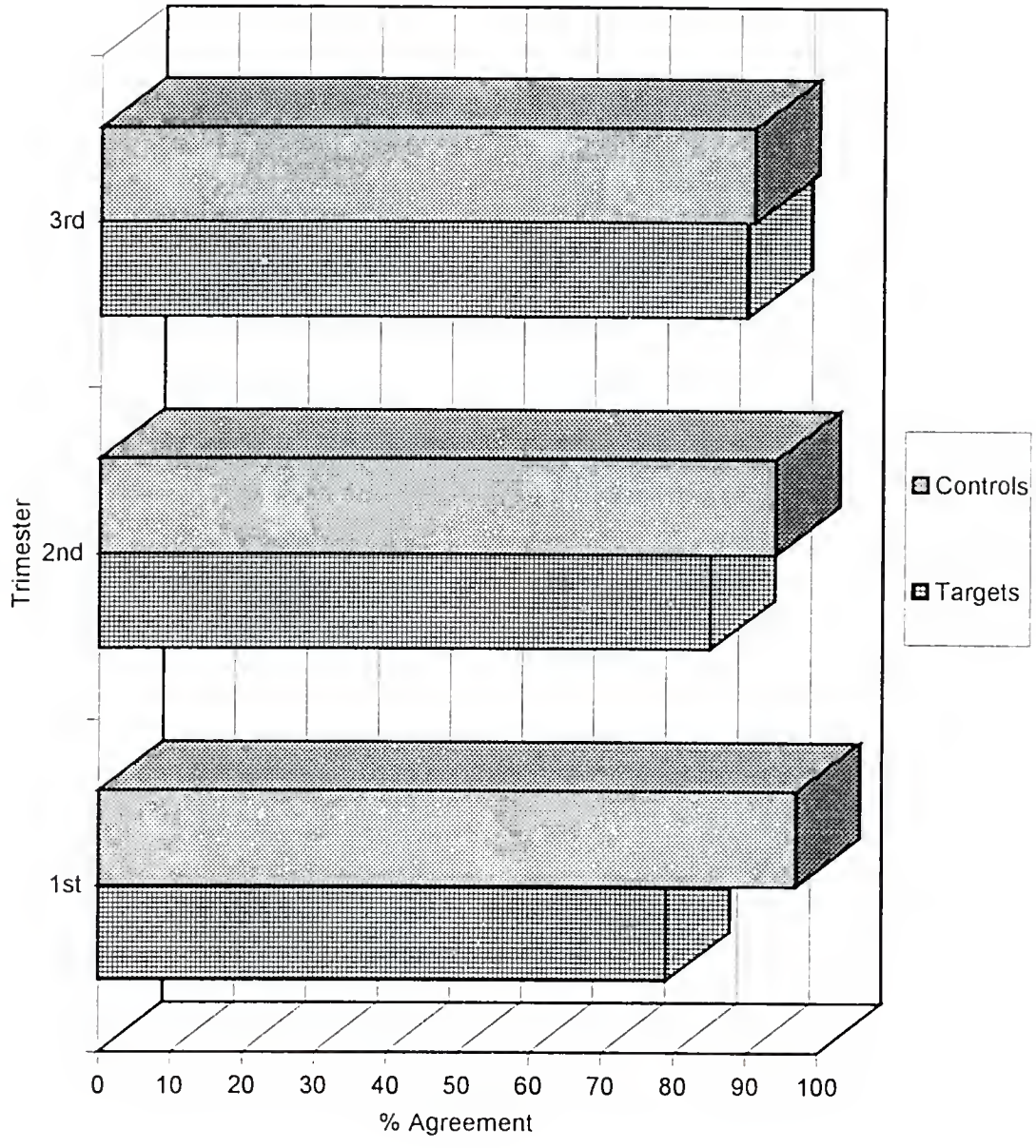


Figure 2-3. Agreement Between Interview and GC/MS Results

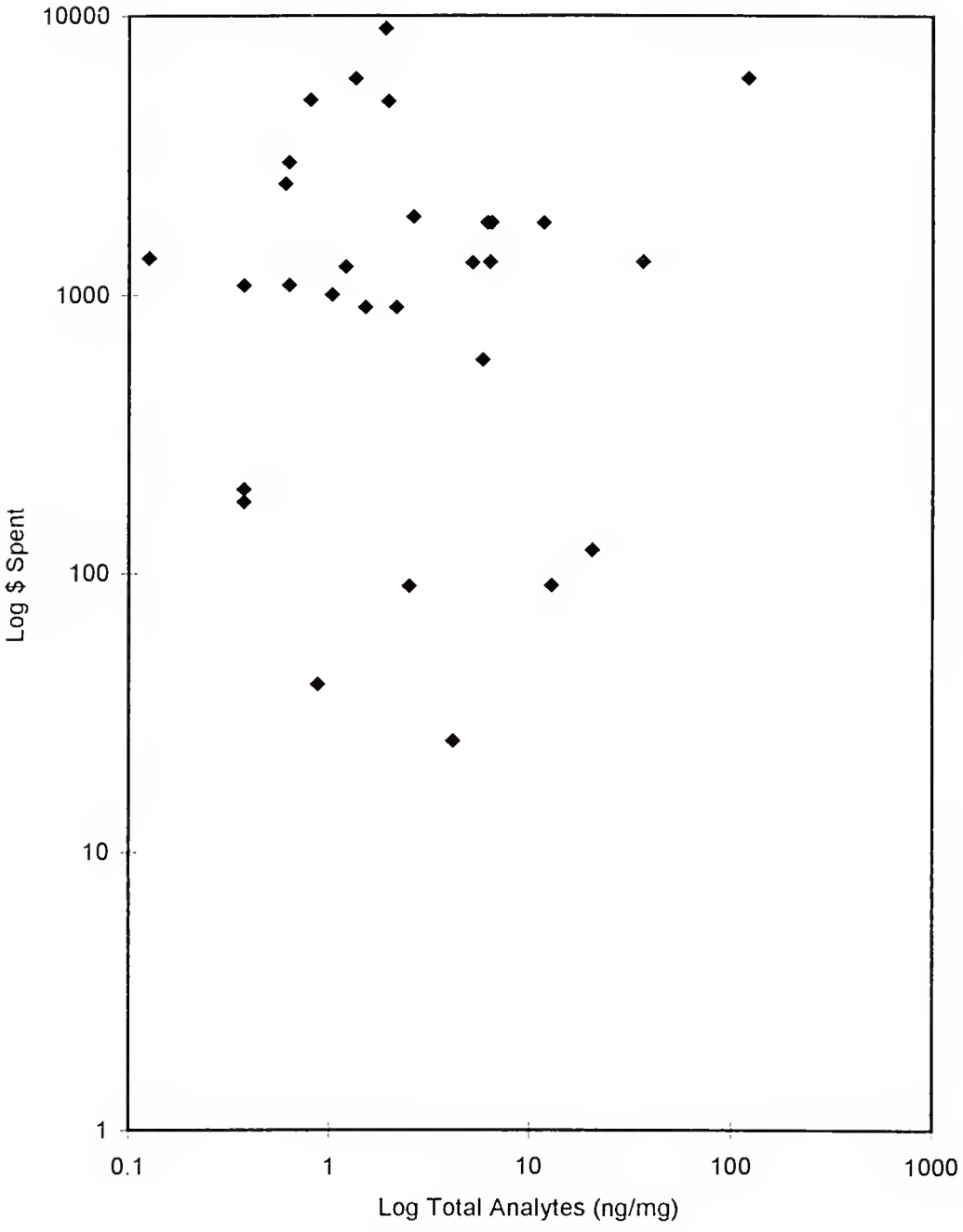


Figure 2-4. Correlation between total amount of analytes found in hair and money spent on cocaine.

Discussion

The SPE and GC/MS methods described in this study differ from those described by Cone et al. in several ways.[27] First, was the addition of the cocaine metabolite, *m*-hydroxybenzoylecgonine, to the assay. Second, was the use of the derivatizing reagent MTBSTFA, which produced higher molecular weight derivatives and improved chromatographic separation than possible with more traditional silyl derivatives. Finally, the volume of elution solvent was increased from 6 to 8 mL. This modified method was found to have excellent sensitivity for the detection of cocaine and its metabolites in hair, and is a satisfactory technique for toxicological testing of this specimen.

The need to investigate new methods of detecting prenatal cocaine exposure is evident by the poor performance of current methods in identifying cocaine exposed infants. This has made research into the effects of gestational cocaine exposure difficult and, consequently, reports have often been unconvincing and contradictory. In some studies, the use of urine and meconium to screen for intrauterine cocaine exposure resulted in false negatives up to 50%.[16,90,91] A possible reason for this is that urine and meconium would not reveal cocaine exposure that occurs during early gestation. It is likely that contradictory reports on the teratogenic effects of cocaine are partially the result

of inaccurate identification of drug users by history or laboratory tests of urine or meconium alone.

In addition, women at highest risk for gestational cocaine use generally receive little or no prenatal care and are therefore unavailable for testing earlier than at delivery.[10] A maternal urine sample collected at this time would only reflect cocaine use within a few days of delivery and result in a high false negative rate.[92] The testing of maternal hair for gestational cocaine abuse has the potential to overcome these limitations. Currently, it is the only specimen that could serve as a biological marker of cocaine use for the entire gestational period. This makes hair particularly useful for identifying cocaine exposure which occurs during the first trimester, where many teratogenic events are believed to take place.[93]

In this study, 56% of first trimester, 88% of second trimester, and 88% of third trimester hair specimens were positive for cocaine analytes from women claiming cocaine use during the respective trimesters. This is lower than the 93.5% for first, 100% for second, and 100% for third trimesters, respectively detected by Callahan et al., the only other report in which maternal hair was segmented into trimesters for analysis.[73] A possible reason for this is that in the study by Callahan et al. hair specimens deemed to be in poor quality were excluded from the study. In this current study no such distinction was made and since poor hair quality can result in enhanced leaching of cocaine from the hair

shaft during normal washing and hygienic practices, the consequence would be lower sensitivity.[85,94]

This same conclusion was also reached by Marques et al., in a study involving hair specimens from 62 mother-infant pairs in which the women had admitted to gestational cocaine use.[85] The authors noted that more than 50% of maternal hair specimens were in poor condition, presumably due to chemical hair treatments. When these specimens were excluded from the data, a significant improvement in the correlation between maternal and infant hair results was noted.[85]

Data from previous studies and the current study are summarized in Table 2-VI. The percentage of positives from subjects admitting to cocaine use ranges from a low of 73% in the current study to a high of 100% in the small study conducted by DiGregorio et al.[86] The high sensitivity reported by DiGregorio et al. is probably the result of the subject selection process, and reflects the purpose of the study which was to look for cocaethylene in maternal hair. Only women who presented for delivery without prenatal care were selected for the study and therefore the study population was probably representative of only the heaviest of drug users. The lower percent positives in the current study was primarily the result of the low sensitivity (56%) of the first trimester specimens as the later trimesters had higher sensitivities (88% each), which are comparable to the percent positives in most of the studies in Table

2-6.

The percentage of positive hair specimens from women denying cocaine use is remarkably similar in all of the studies except for the 1993 study conducted by DiGregorio et al.[86] This is probably due to the same reasons for the high sensitivity among positives in the DiGregorio et al. study as outlined above, and the fact that only five subjects denying cocaine use were tested from this highly suspect population.

The average ratio of cocaine to benzoylecgonine was 5.7 which is the same ratio calculated from the DiGregorio et al., 1994 study, and is comparable to cocaine/benzoylecgonine ratios of 5-9, reported elsewhere.[81,87] Seven specimens from this current study were positive for benzoylecgonine only, which is not consistent with published hair data, in which cocaine is the analyte most often detected.[81,87] Of the studies outlined in Table 2-VI, only the two DiGregorio et al. studies and the current study included analytes other than cocaine. All positive hair specimens in the two DiGregorio et al. studies contained cocaine and benzoylecgonine.[86,87] Maternal drug histories were not included in the published data from the DiGregrio et al. studies, but all subjects were specifically recruited from women who were admitted to labor and delivery without prenatal care.[86,87] This would make the subjects more likely to be heavy and recent drug users.[10]

In the current study, the specimens containing only benzoylecgonine came from subjects whose average number of days since last cocaine use were 129 (range: 41-236). In contrast, the specimens containing cocaine had maternal histories whose average number of days since last use were 16 (range: 0-92). The half-life of cocaine in hair is reported to be between 1.2 and 2.1 months which is shorter than the half-life of benzoylecgonine. [79] This large variation in half-life is expected because, the length of time that cocaine analytes can be detected in hair is heavily dependent on the frequency of hair washing and chemical treatments.[79] Moreover, the use of chemical coloring, permanent waving, and straightening agents which use basic solutions to achieve desired results, may cause an accelerated conversion of cocaine to benzoylecgonine in the hair of individuals who use these products.[79] Cosmetic hair treatments can also damage the hair shaft. Studies have shown that cosmetically treated hair is more likely to leach drugs at a higher rate than healthy hair because of damage to the cuticle and because cosmetic treatments themselves, extract drugs from hair.[79] Since the current study did not include a history of hair washing and cosmetic treatments, it is difficult to speculate as to the true cause of the specimens that tested positive for benzoylecgonine only, but the reason may be due to any of the factors mentioned above or any combination thereof.

An important concern regarding the use of hair analysis to determine cocaine abuse, is the issue of environmental contamination of hair with cocaine.

In this study, methanol was chosen as the wash solvent because in previous studies evaluating the efficacy of various wash methods for decontaminating hair, methanol was found to be the most effective for removing externally applied drug.[88,95] Thirteen percent of all wash specimens were positive for one or more cocaine analytes in this study. All but one of these positive wash specimens were from women who admitted to the use of cocaine during their pregnancy. Among the five controls whose hair specimens were positive for cocaine analytes, all of the wash specimens were negative and three of the five also had corroborating positives in other specimens tested, such as infant urine, meconium or amniotic fluid. The one positive wash specimen from a control subject, had a corresponding positive infant urine specimen but a negative hair specimen.

Maternal drug use histories are reported to be unreliable because of social and legal repercussions that can come with admitting to drug use.[10,14] The good correlation between maternal drug use history and qualitative hair results in this study is because the women recruited as targets for this study had already been identified by hospital staff as drug users and would not suffer any additional consequences for supplying the interviewer with information concerning their drug use. The lack of correlation between amounts of drug use reported and quantitative hair results is not surprising. Other researchers have reported a lack of correlation between quantitative results and reported drug use

and as discussed earlier the correlation improved when specimens with poor hair quality were excluded from the analysis.[85,94]

This study has shown that maternal hair analysis for drugs can give useful information about the qualitative use of drugs during pregnancy especially for use in the realm of health outcome of those infants exposed to drugs during gestation. With larger and more comprehensive studies, hair testing may answer questions about the consequences of prenatal cocaine exposure where serial urine sampling is not a practical alternative because of its cost and inconvenience. This is consistent with the view that, over time drug analytes can leach out of hair. It is not known what implications this might have on the efficacy of maternal hair in detecting drug use that is sporadic or drug use that occurs only in early pregnancy.

CHAPTER 3
QUANTITATIVE DETERMINATION OF COCAINE AND ITS METABOLITES IN AMNIOTIC FLUID, COLOSTRUM AND UMBILICAL CORD TISSUE

Introduction

Cocaine use by pregnant women has become a public health tragedy, particularly in urban areas where surveys have shown an increase in the number of newborns testing positive for cocaine and other drugs.[9,16,42] Immunoassay is the usual method for detection of drug use during pregnancy and is typically performed on urine obtained from either the mother or infant. In addition, meconium has recently been used to detect gestational cocaine exposure with varying amounts of success.[13,74,96] Both methods have reported false negative rates between 22% and 60%, and consequently fall short of ideal at detecting prenatal cocaine exposure.[13,16,56,73,74,96] Alternative specimens such as amniotic fluid, umbilical cord tissue, and colostrum have not been as thoroughly studied as have meconium and urine. Therefore, an investigation of these specimens is an important step in improving the identification of cocaine exposed neonates. The purpose of this work was to describe the extraction and detection of cocaine and its metabolites in three rarely used specimens; amniotic fluid, colostrum, and umbilical cord tissue.

Amniotic fluid is present throughout gestation, and it has been suggested that large amounts of cocaine and its metabolites may accumulate in this fluid. The fetus would thus be exposed, both orally and transdermally, to the drug and metabolites during gestation.[40,91,97] Umbilical cord tissue is also present throughout gestation, but there has been little data published regarding this tissue.[97]

Amniotic fluid forms initially when the amniotic cells secrete a small amount of fluid into the amniotic sac, but this is quickly replaced by maternal interstitial fluid which diffuses across the amniochorionic membrane.[93] By week ten of gestation, the majority of amniotic fluid is the result of diffusion of water and solutes from blood in the intervillous space of the placenta. In addition, before keratinization of the skin takes place between week 22 and 25 of gestation, large amounts of interstitial water and solutes from fetal tissue easily diffuse through the fetal skin into the amniotic cavity.[98] Beginning in week 11, fetal urine is expelled into the amniotic cavity and by the end of gestation, fetal urine volumes average 500 mL per day.[93]

Amniotic fluid was once considered to be a stagnant pool, but this view has changed with the advent of amniotic fluid studies using isotopically labeled water.[98] It is now known that the water content of amniotic fluid changes every three hours.[93] Amniotic fluid is said to be in balance with the fetal circulation because an exchange of fluid with fetal blood occurs through the umbilical cord

and where the amniotic membrane adheres to the surface of the placenta. In addition, amniotic fluid is absorbed by the fetus's respiratory and digestive tracts following fetal swallowing of the amniotic fluid. The fluid is then absorbed into the fetal blood stream and passes into the maternal circulation via the placenta. Any excess water in the fetal blood is then excreted into the amniotic sac in the form of urine by the fetal kidneys.[93]

In the first trimester, amniotic fluid has an osmolality and electrolyte composition that is basically the same as fetal blood.[98] The fluid can be said to be a solution of approximately 99% water in which salts and undissolved material such as epithelial cells, proteins, hormones and pigments are suspended.[93] Later in gestation, and after keratinization of the skin takes place, the composition of amniotic fluid begins to change as fetal urine contributes a greater percentage to the total volume.[98] Maternal plasma also contributes to amniotic fluid in the form of proteins, glucose and enzymes. The fetal lungs contribute fluid and lipids to the amniotic fluid as surfactants are formed to prepare the lungs for birth.[99] In addition to water, fetal urination contributes trace elements, urea, creatinine, uric acid, free amino acids, organic acids, triglycerides, hormones, enzymes, and other metabolic products or substances passed from the mother to the fetus such as drugs.[99] It is because fetal urine contributes waste substances like drugs and metabolites to amniotic fluid, that

testing of amniotic fluid for drugs of abuse is a valid alternative to the use of neonatal urine.

Colostrum, the thin, yellow, milky fluid (87% water) is secreted by the mammary gland a few days before and after parturition. Colostrum is composed of more than 100 known constituents, including 8% protein, 1% fat, 3% carbohydrate, and 1% mineral content. The protein composition includes albumin, IgA, lactoferrin, α-lactoalbumin and colostrum corpuscles. The percentage of fat and carbohydrate are one-third and one half, respectively, of that found in mature breast milk. In addition, the mineral content of colostrum is three times higher than that of mature breast milk.[98] A large amount of research has been done on the excretion of prescription and over the counter drugs in mature breast milk.[100] Additionally, researchers have investigated the excretion of a few drugs of abuse into mature breast milk, but, there does not appear to be any published reports of cocaine and metabolites in colostrum.[101] The comprehensive analysis of cocaine and its metabolites in amniotic fluid, colostrum, umbilical cord tissue, and perhaps other maternal/fetal fluids and tissues, may help further characterize neonatal drug exposure.

This chapter presents a method for determining cocaine and its metabolites in amniotic fluid, colostrum and umbilical cord tissue from subjects. In addition, data from these specimens and the possible implications of detecting drugs in these specimen types are discussed.

Materials and Methods

Subjects

Subjects admitting to cocaine use during their current pregnancy were asked to participate in this study as a target (n=34). Women admitted to labor and delivery that were not known to be cocaine users were recruited to participate as controls (n=49). Participants were interviewed confidentially to assess the amount and timing of drug use during each trimester.

Specimens

All biological specimens were marked with a unique research identification number and the analyst was blinded to drug history. Five mL of amniotic fluid was collected from 32 subjects during parturition. A random three centimeter segment of umbilical cord from 70 subjects was collected immediately following parturition and placed in a sterile container. Maternal subjects were also encouraged to provide a colostrum sample, and for those that agreed (n=21), the specimen was placed in a sterile container. All specimens were stored at -20°C until analysis.

Chemicals

Benzoylecgonine (BE), cocaine (COC), ecgonine methyl ester (EME), cocaethylene (CE), norcocaine (NCOC), and trideuterated analogs for BE, EME, COC, and CE were purchased from Radian Corporation (Austin, TX). Ecgonine ethyl ester (EEE) and *m*-hydroxybenzoylecgonine (MOHBE) were a generous gift from Edward Cone, Ph.D. Methyl-(tert-butyldimethylsilyl)-trifluoracetamide (MTBSTFA) was purchased from Pierce Chemical Company (Rockford, IL). All other reagents were of reagent grade and were purchased from Fisher Scientific (Orlando, FL).

Standard preparation

Calibration standards were prepared by fortifying the appropriate blank specimen type with aliquots of standard solutions to form a calibration curve in the range of 25-750 ng/mL or ng/g. Control specimens prepared at 0, 200, and 600 ng/mL, or ng/g, for all analytes were included in each analytical run. In addition, a 500 ng/mL cocaine standard was included in each run to monitor hydrolysis of COC to BE and/or EME during extraction and subsequent analysis. All standards and controls were prepared immediately prior to extraction.

Extraction of Biological Matrices

The solid phase extraction (SPE) of cocaine and its metabolites and subsequent analysis by GC/MS was adapted from the procedure previously described by Cone et al. [27]

Umbilical Cord Tissue: To 1 g of umbilical cord tissue, bupivacaine (1 μg) was added as an internal standard. The tissue was homogenized with 3 mL of phosphate buffer (0.025M, pH 4) using an Eberbach ConTorque homogenizer (Ann Arbor, MI). Homogenates were centrifuged for 10 min at 1000 x g, and the supernatants were decanted and applied to CleanScreen™ SPE extraction columns (ZSDAU020; United Chemical Technologies, Horsham, PA) that had previously been conditioned with elution solvent (1 x 1 mL), methanol (1 x 3 mL), deionized water (1 x 3 mL), and 0.025M (pH 4) phosphate buffer (1 x 2 mL). The specimens were followed with a wash of deionized water (1 x 2 mL) and 0.1M HCl (1 x 2 mL). The columns were then air dried at full vacuum for 2 min. A methanol wash (1 x 6 mL) and a second 2 min. drying step completed the washes. The analytes were then collected in culture tubes by eluting with 8 mL of the elution solvent (methylene chloride: isopropanol: concentrated aqueous ammonium hydroxide, 80:20:2 by vol). The extracts were evaporated to dryness at 40°C under a gentle nitrogen stream. The extracts were derivatized by adding MTBSTFA (30 μL) and heating at 90°C for 60 min.

Colostrum: Deuterated analogs of cocaine and metabolites as internal standard were added to 1 mL of colostrum to achieve a concentration of 200 ng/mL followed by 3 mL of phosphate buffer (0.025M, pH 4). The specimens were then applied to the CleanScreen™ SPE extraction columns in the same manner as described above.

Amniotic fluid: The viscosity of amniotic fluid required the use of the high flow X-TracT SPE column (XRDAH515; United Chemical Technologies, Horsham, PA) for the extraction procedure. To 1 mL of amniotic fluid, bupivacaine (1.0 μg) was added as an internal standard, followed by 3 mL of phosphate buffer (0.025M, pH 4). The specimens were then applied to the X-TracT columns in the same manner as described above.

Gas Chromatography/ Mass Spectrometry (GC/MS)

GC/MS analyses were performed with a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a Hewlett-Packard 7673A automated liquid sampler and interfaced with a Hewlett-Packard 5972A mass selective detector. The GC and detector were controlled by a Hewlett-Packard data system. The GC column was a HP-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness). Helium was the carrier gas, programmed at a constant flow rate of 1 mL/min. The injection port was fitted with a 2-mm silanized glass liner. The injection port temperature was 275°C, and the detector temperature was 290°C.

The initial oven temperature of 90°C was maintained for 1 min, programmed to 220°C at 30°C /min and held for 0.5 min, then programmed to 330°C at 20°C /min and maintained for 1 min. The total run time was 12.33 min. The GC/MS was operated in the selected ion monitoring (SIM) mode with a dwell time of 20 ms/ion and quantitation was based upon ion peak area ratios of analyte to internal standard. The quantitation and identification ions for each analyte and internal standard are listed in Table 3-1.

Results

The calibration curves for each analyte in all specimen types were found to be linear over the calibration range by use of the standard error of the estimate statistic (Appendix I. Curves I-22 to I-42). Limit of detection (LOD) was defined as the concentration corresponding to a signal to noise ratio of 3. The limit of quantitation (LOQ) was determined by the analysis of a series of decreasing standards and defined as the lowest standard that did not deviate from the target concentration by more than 20%. Recovery and precision were measured at two concentrations, level one at 100 ng/mL or ng/g and level two at 500 ng/mL or ng/g. The CV's for several analytes were at the high end of the acceptable range and was probably due to low intensity of the quantitation ion

and low recovery. The LOD, LOQ, recovery, and precision for each analyte in each specimen type are presented in Table 3-2,A-C.

Figure 3-1,A-D illustrates a SIM chromatogram of an unextracted standard (A), a 500 ng/mL extracted standard (B), a negative quality control specimen (C) and a positive amniotic fluid specimen obtained from a target subject (D). The target specimen was found to contain benzoylecgonine (152,288 ng/mL), ecgonine methyl ester (11,879 ng/mL), ecgonine ethyl ester (335 ng/mL) and trace amounts of cocaine and *m*-hydroxybenzoylecgonine.

Table 3-3 summarizes the range of analyte concentrations found in amniotic fluid, colostrum, and umbilical cord tissue. In addition, the number of positives in each category is presented. Cocaine and cocaine metabolite results for each positive specimen in amniotic fluid, colostrum, and umbilical cord tissue are presented in Figure 3-2, A-C. In general, amniotic fluid contained the highest concentrations of analytes, with benzoylecgonine being the most common analyte detected, followed by ecgonine methyl ester. In colostrum, the most common analyte detected was cocaine, followed by benzoylecgonine. In umbilical cord tissue, benzoylecgonine was the most common analyte, followed by ecgonine methyl ester. Trace amounts of cocaine, norcocaine, and *m*-hydroxybenzoylecgonine were found in amniotic fluid, and one specimen was positive for ecgonine ethyl ester. In umbilical cord tissue, small amounts of ecgonine methyl ester, cocaine, and *m*-hydroxybenzoylecgonine were detected,

and in colostrum, small amounts of norcocaine and ecgonine methyl ester were detected. Cocaethylene was not detected in any specimen type.

Table 3-1. Cocaine Analyte and Internal Standard Target and Qualifier Ions

Analyte	Target Ion (m/z)	Qualifier Ions (m/z)
Ecgonine methyl ester	256	182, 282
Ecgonine ethyl ester	270	196, 282
Norcocaine	168	289
Cocaine	182	272, 303
Cocaethylene	196	272, 317
Benzoylecgonine	346	282, 403
m-hydroxybenzoylecgonine	282	533, 82
Bupivacaine	141	140, 84
d3-Ecgonine methyl ester	185	259, 285
d3-Cocaine	306	275
d3-Cocaethylene	199	275, 320
d3-Benzoylecgonine	349	285, 406

Table 3-2A. Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery, and Precision of Cocaine Analytes in Amniotic Fluid*

Analyte	LOD*	LOQ*	Recovery (%)		Within Run Precision (%)		Between Run Precision (%)	
			Level 1**	Level 2**	Level 1	Level 2	Level 1	Level 2
EME	5	10	76	76	11	19	9	16
EEE	50	50	80	77	22	19	18	23
NCOC	5	10	85	93	10	9	16	12
COC	5	10	117	96	8	8	15	15
CE	5	10	110	121	9	6	13	12
BE	5	10	120	120	10	11	12	14
MOHBE	10	25	76	89	16	18	16	22

Table 3-2B. Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery, and Precision of Cocaine Analytes in Umbilical Cord Tissue*

Analyte	LOD*	LOQ*	Recovery (%)		Within Run Precision (%)		Between Run Precision (%)	
			Level 1**	Level 2**	Level 1	Level 2	Level 1	Level 2
EME	5	10	73	106	19	10	16	18
EEE	25	50	70	109	26	7	21	13
NCOC	10	25	84	83	10	9	13	16
COC	2.5	5	74	101	9	8	11	9
CE	2.5	5	72	91	9	12	8	15
BE	2.5	2.5	71	108	7	8	11	13
MOHBE	5	10	107	112	5	8	8	12

Table 3-2C. Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery, and Precision of Cocaine and Metabolites in Colostrum*

Analyte	LOD*	LOQ*	Recovery (%)		Within Run Precision (%)		Between Run Precision (%)	
			Level 1**	Level 2**	Level 1	Level 2	Level 1	Level 2
EME	2.5	5	63	71	16	11	19	19
EEE	25	50	41	53	23	22	28	19
NCOC	5	10	49	58	8	10	10	9
COC	2.5	10	100	108	9	8	9	8
CE	5	10	78	83	9	9	9	12
BE	2.5	5	82	89	11	10	13	11
MOHBE	10	25	52	64	8	16	14	18

*Concentrations noted in ng/mL (amniotic fluid and colostrum) or ng/g (cord tissue).
** Level 1 is 100 ng/mL or ng/g and Level 2 is 500 ng/mL or ng/g.
Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 3-3. Concentration Range of Cocaine Analytes in Amniotic Fluid, Colostrum and Umbilical Cord Tissue and Number of Positive Specimens in Each Subject Category.

Matrix	Total number of subjects	EME	EEE	NCOC	COC	CE	BE	MOHBE
Amniotic Fluid (ng/mL)		0-11,800	0-335	0-Trace	0-Trace	0	0-152,200	0-Trace
Controls	19	1	0	0	0	0	2	0
Targets	13	4	1	1	1	0	7	3
Total*	32	5	1	1	1	0	9	3
Cord Tissue (ng/g)		0-52	0	0-172	0-Trace	0	0-1237	0-Trace
Controls	42	0	0	1	0	0	1	0
Targets	28	5	0	2	1	0	12	3
Total*	70	5	0	3	1	0	13	3
Colostrum (ng/mL)		0-119	0	0-Trace	0-12,130	0	0-4070	0
Controls	11	4	0	1	6	0	4	0
Targets	10	0	0	0	0	0	0	0
Total*	21	4	0	1	6	0	4	0

*Although 83 total subjects were recruited, amniotic fluid, colostrum and umbilical cord tissue specimens were not available for every subject.

Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 3-4. Summary of Cocaine Analyte Concentrations (ng/mL) Reported in the Literature and the Present Study

INVESTIGATOR	CASE	COC	BE	EME	OTHERS
Moore et al. 1992	1	Trace	230	NA	
	2	Trace	40	NA	
	3	250	3060	NA	
	4	ND	1980	NA	
	5	ND	1650	NA	
	6	ND	750	NA	
	7	14	390	NA	
	8	70	290	NA	
Ripple et al. 1992	1	18	836	34	
	2	Trace	113	Trace	
	3	11	277	11	Trace CE
	4	19	552	17	
	5	24	51	Trace	
Casanova et al. 1994	1	ND	909	115	
	2	ND	925	40	
	3	ND	143	ND	
Current Study	4	ND	Trace	ND	
	14	ND	Trace	ND	
	6	ND	Trace	Trace	
	73	ND	Trace	ND	
	10	ND	91	ND	
	13	ND	339	Trace	Trace NCOC
	25	ND	555	104	Trace MOHBE
	61	ND	1435	115	Trace MOHBE
	56	Trace	152288	11879	EEE 339, Trace MOHBE

Abbreviations are as follows: ND = not detected; NA = not analyzed; COC = cocaine; BE = benzoylecgonine; EME = ecgonine methyl ester, MOHBE= *m*-hydroxybenzoylecgonine, EEE=ecgonine ethyl ester, CE=cocaethylene

Figure 3-1A. Total ion chromatogram from an Unextracted Standard. Internal Standard: bupivacaine (BUPI) Analytes: Ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), *m*-hydroxybenzoylecgonine (MOHBE) are readily detected in the standard.

File : C:\HPCHEM\1\DATA\WINECKER\03OCT95A.01P\001SP001.D
Operator : R. WINECKER
Acquired : 3 Oct 95 1:32 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: UNEXTRACTED STANDARD
Misc Info :
Vial Number: 1

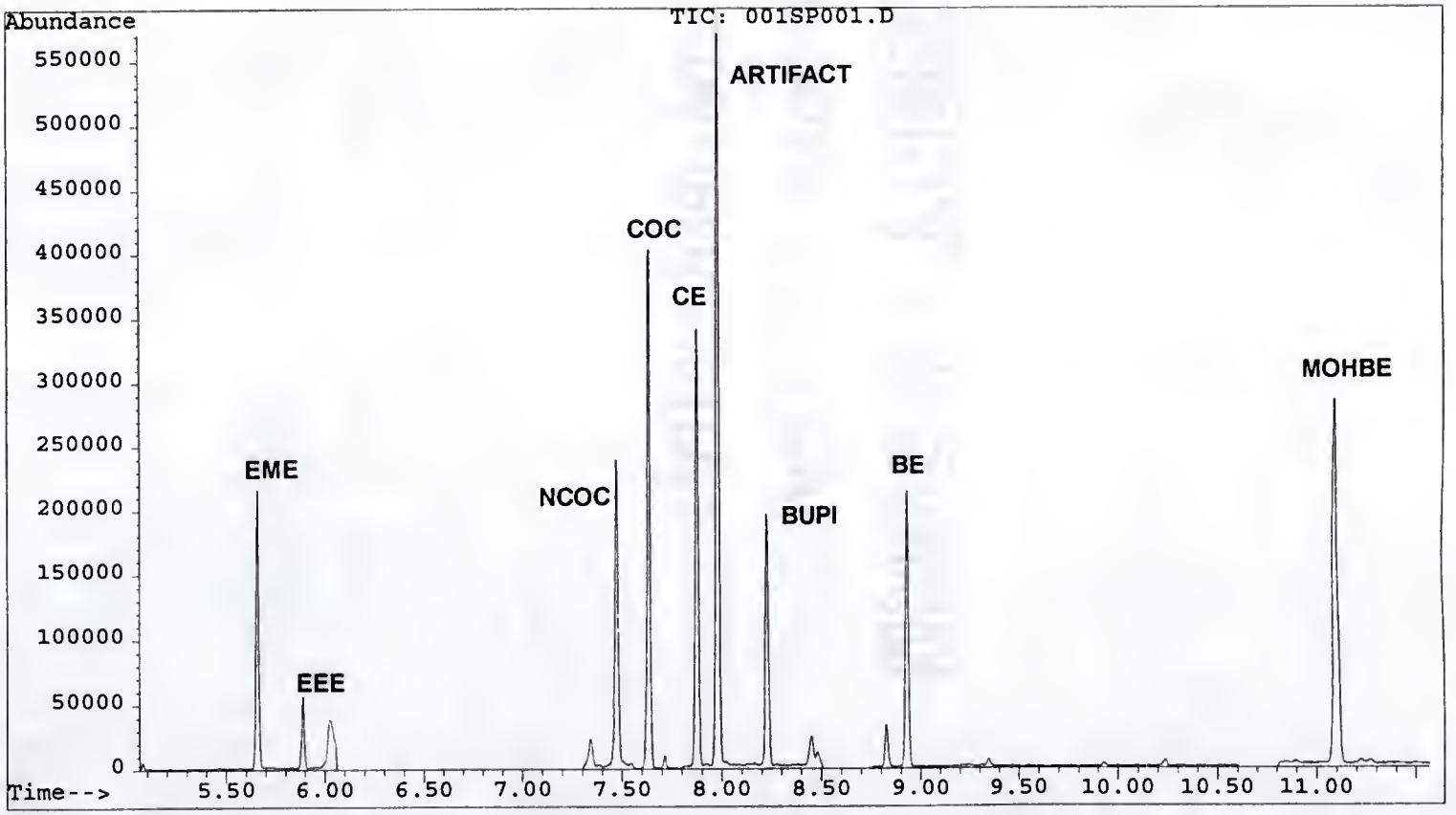


Figure 3-1B. Total ion chromatogram of an extract from a 500 ng/mL extracted standard. Internal Standard: Bupivacaine (BUPI) Analytes: Ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), *m*-hydroxybenzoylecgonine (MOHBE) are readily detected in the standard.

File : C:\HPCHEM\1\DATA\WINECKER\03OCT95A.01P\002SP002.D
Operator : R. WINECKER
Acquired : 3 Oct 95 1:49 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: 500 NG/ML
Misc Info :
Vial Number: 2

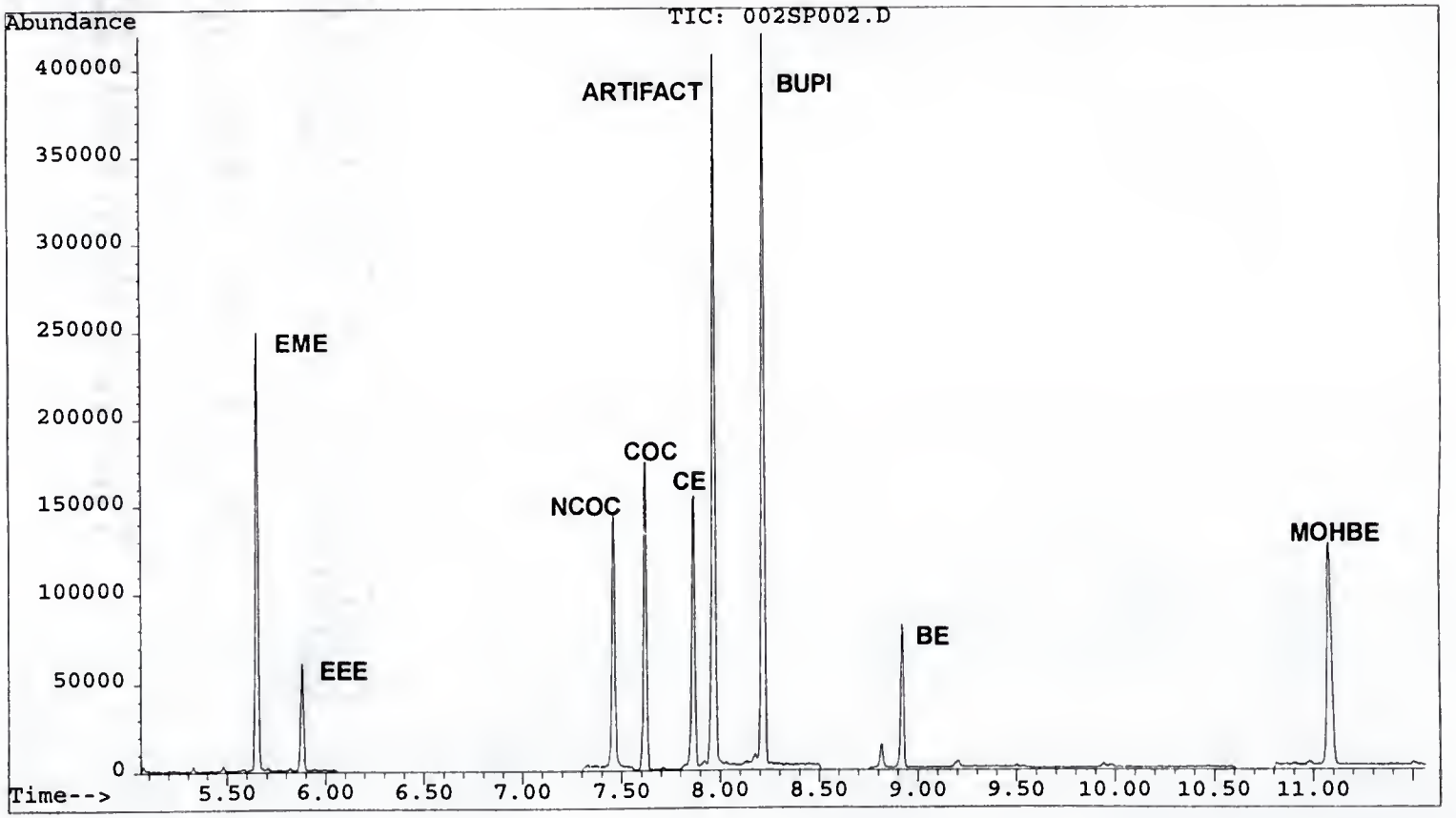


Figure 3-1C. Total ion chromatogram of an extract from a negative quality control standard. Internal Standard: Bupivacaine (BUPI).

File : C:\HPCHEM\1\DATA\WINECKER\03OCT95A.01P\008SP008.D
Operator : R. WINECKER
Acquired : 3 Oct 95 3:28 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: NEG
Misc Info :
Vial Number: 8

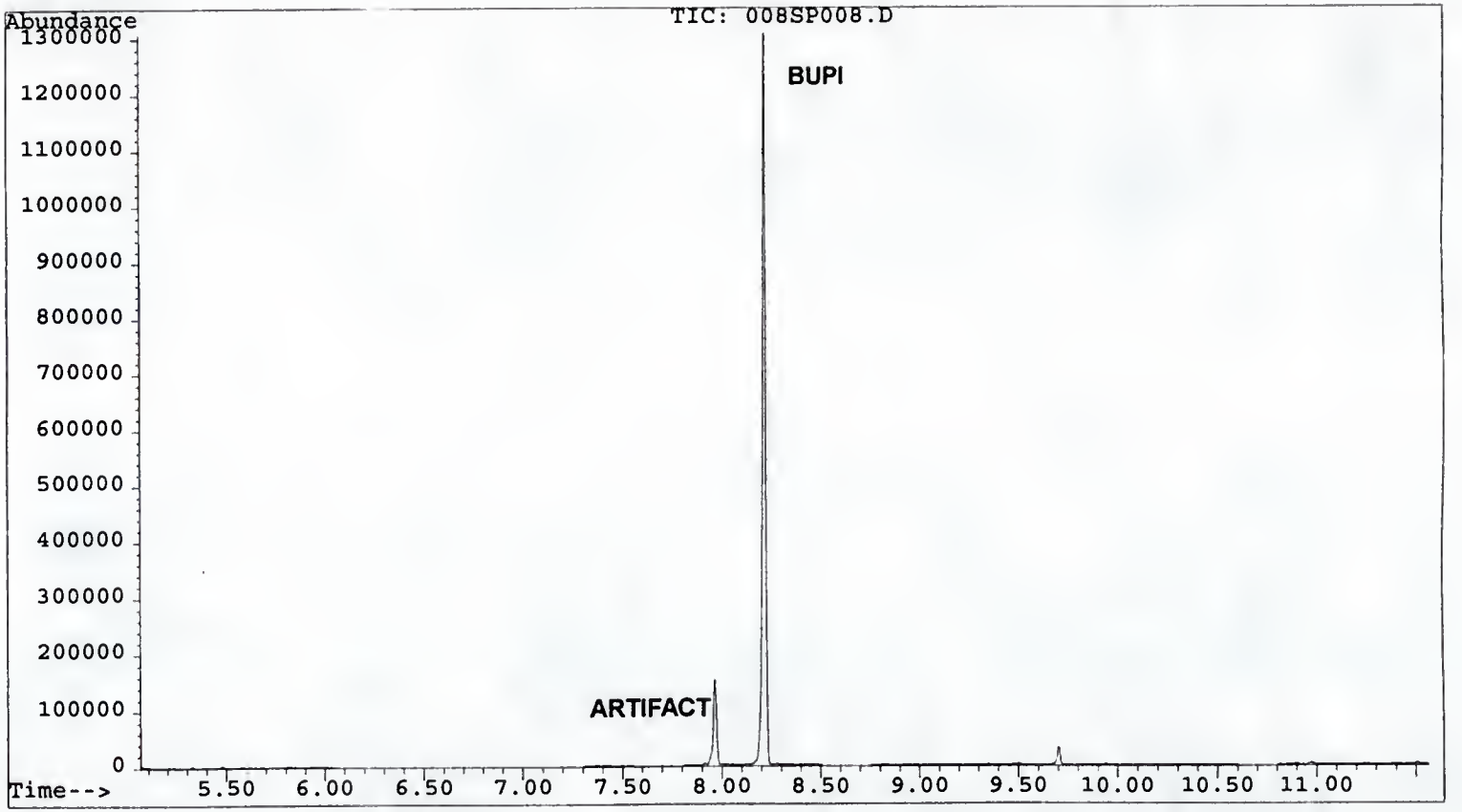


Figure 3-1D. Total ion chromatogram of an extract from a positive amniotic fluid specimen obtained from a target subject. Internal Standard: Bupivacaine (BUPI) Analytes: The target specimen was found to contain benzoylecgonine (152,288 ng/mL), ecgonine methyl ester (11,879 ng/mL), ecgonine ethyl ester (335 ng/mL) and trace amounts of cocaine and *m*-hydroxybenzoylecgonine.

File : C:\HPCHEM\1\DATA\WINECKER\02OCT95A.05P\061SP042.D
Operator : R. WINECKER
Acquired : 3 Oct 95 10:04 am using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: CS1056
Misc Info :
Vial Number: 42

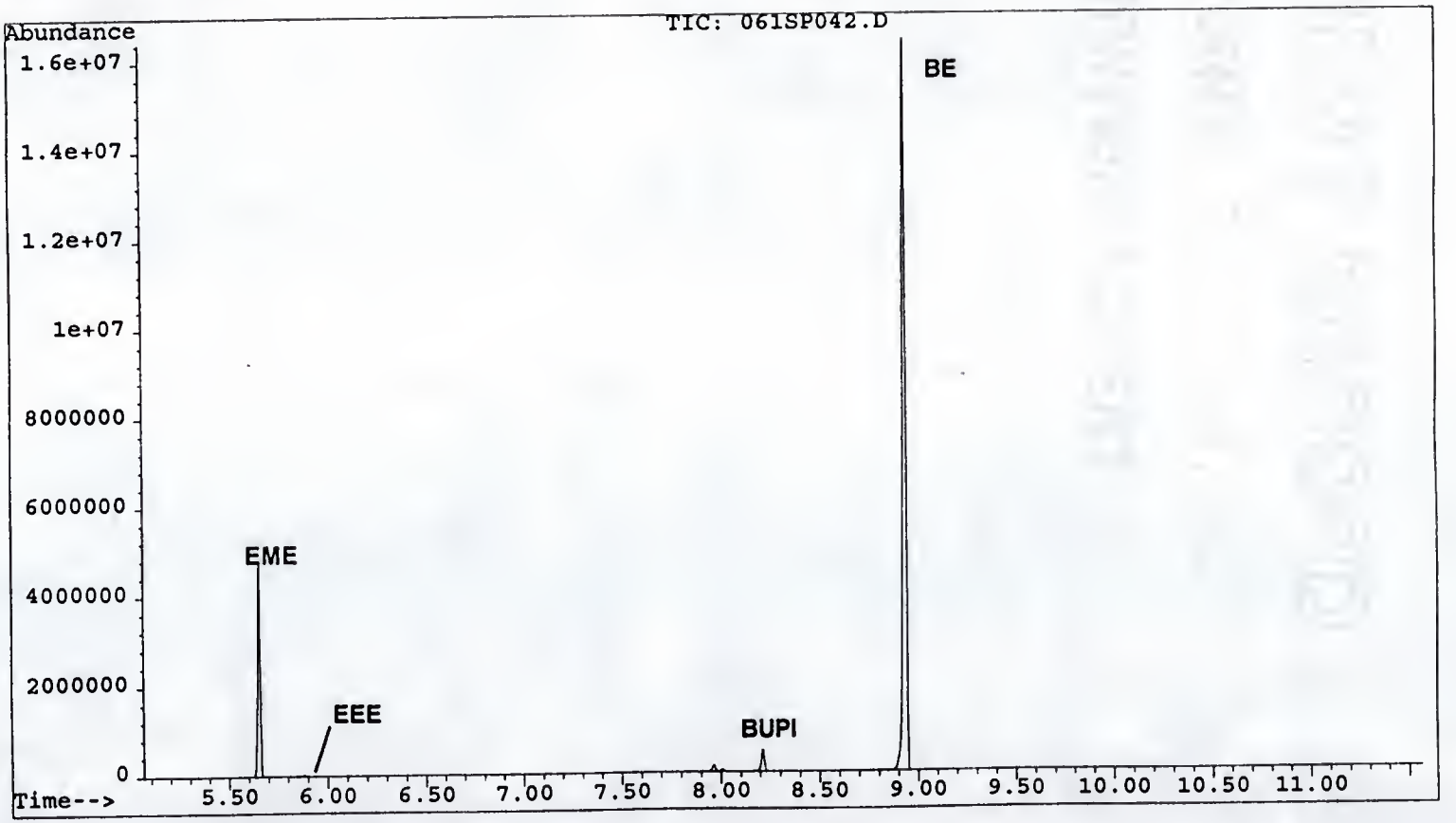


Figure 3-2A. Cocaine analyte results for all positive specimens in amniotic fluid. Note: The large variation in quantitative results required conversion of the raw data to log values in order to provide meaningful graphs. All values below log 10 ng/mL are less than the limit of quantitation and are designated as trace.

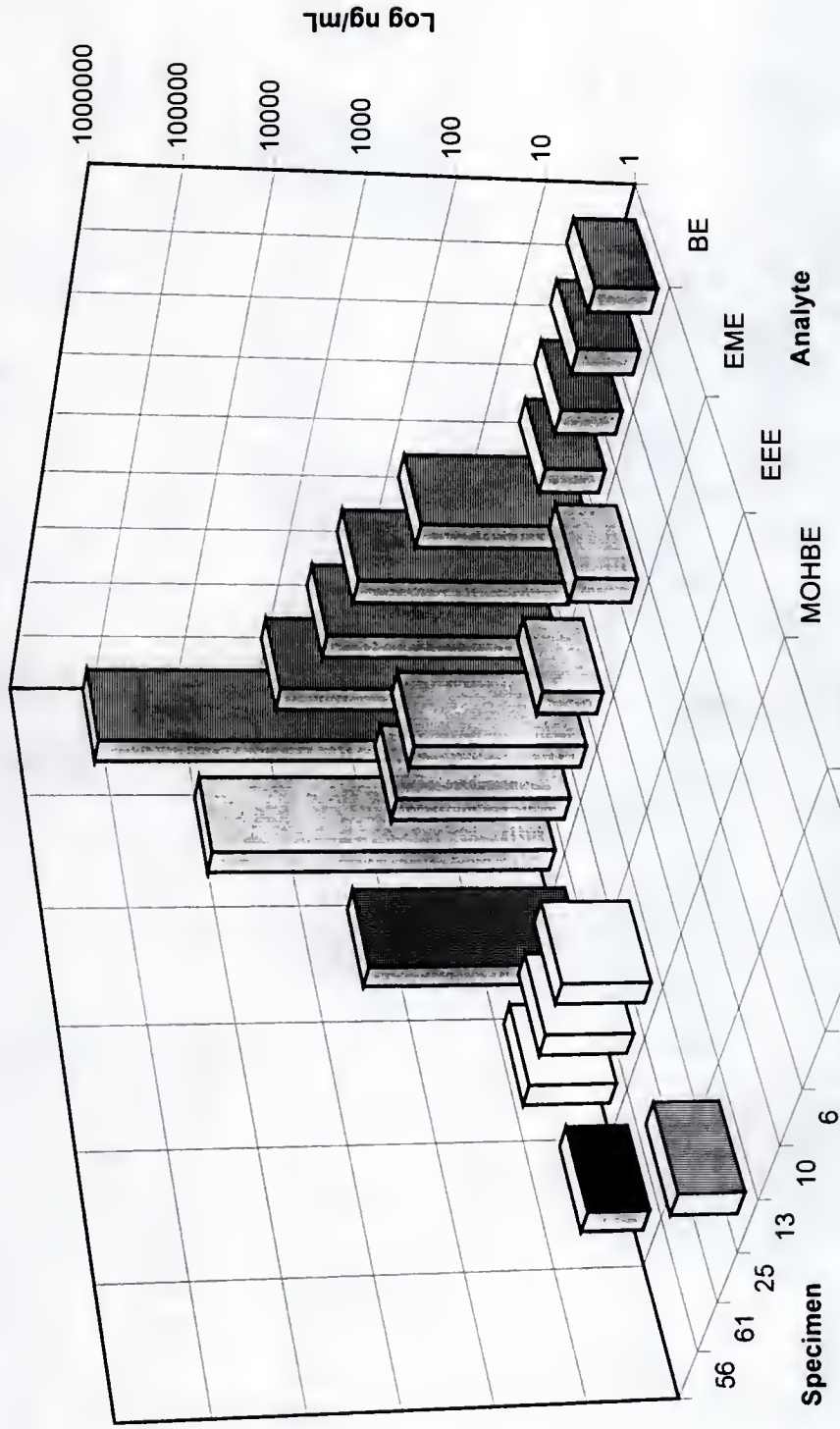


Figure 3-2A. Cocaine analyte results for positive amniotic fluid specimens.

Figure 3-2B. Cocaine analyte results for all positive specimens in Umbilical cord tissue. Note: The large variation in quantitative results required conversion of the raw data to log values in order to provide meaningful graphs. All values below log 10 ng/g are less than the limit of quantitation and are designated as trace.

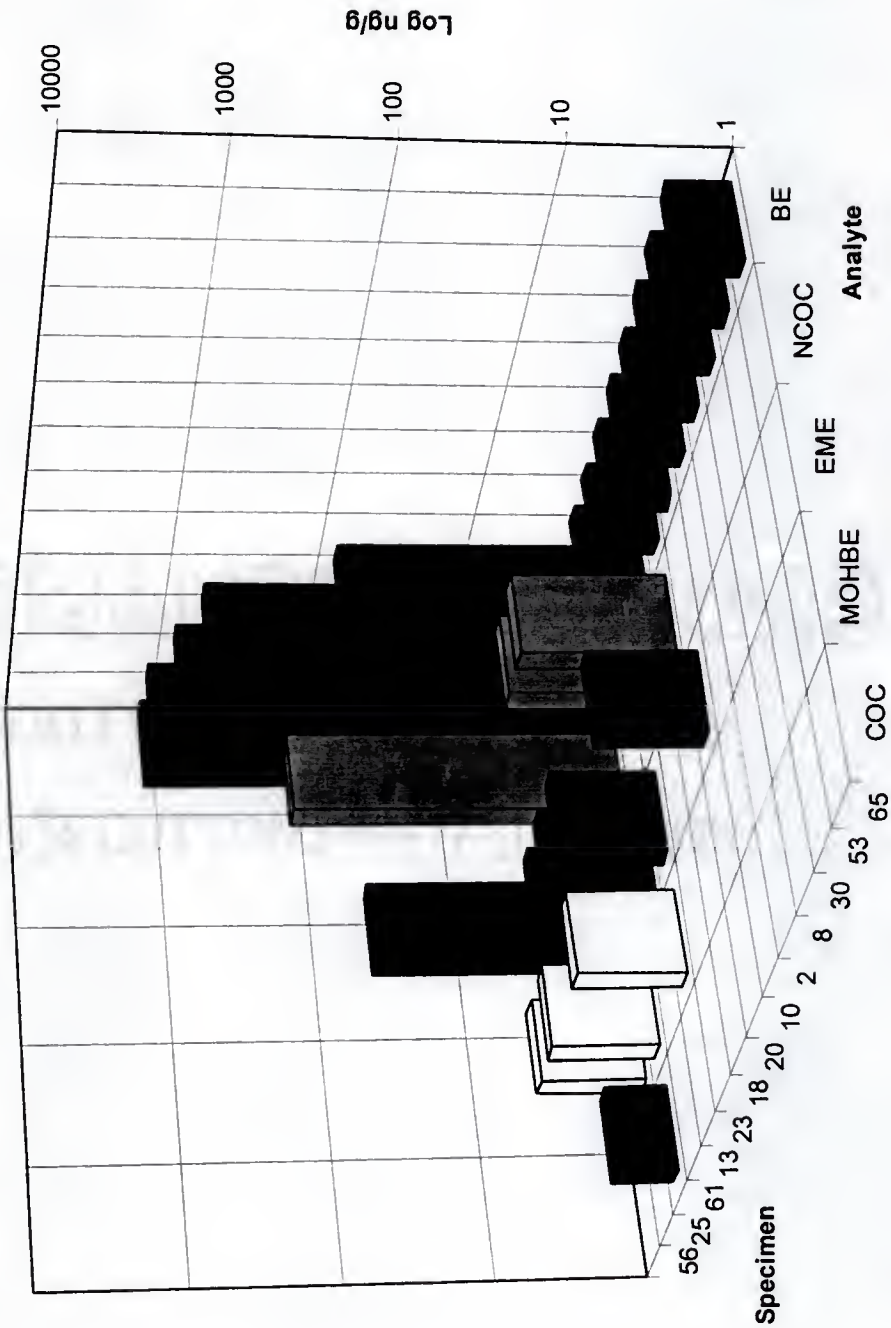


Figure 3-2B. Cocaine analyte results for positive umbilical cord tissue specimens.

Figure 3-2C. Cocaine analyte results for all positive specimens in colostrum. Note: The large variation in quantitative results required conversion of the raw data to log values in order to provide meaningful graphs. All values below log 10 ng/mL are less than the limit of quantitation and are designated as trace.

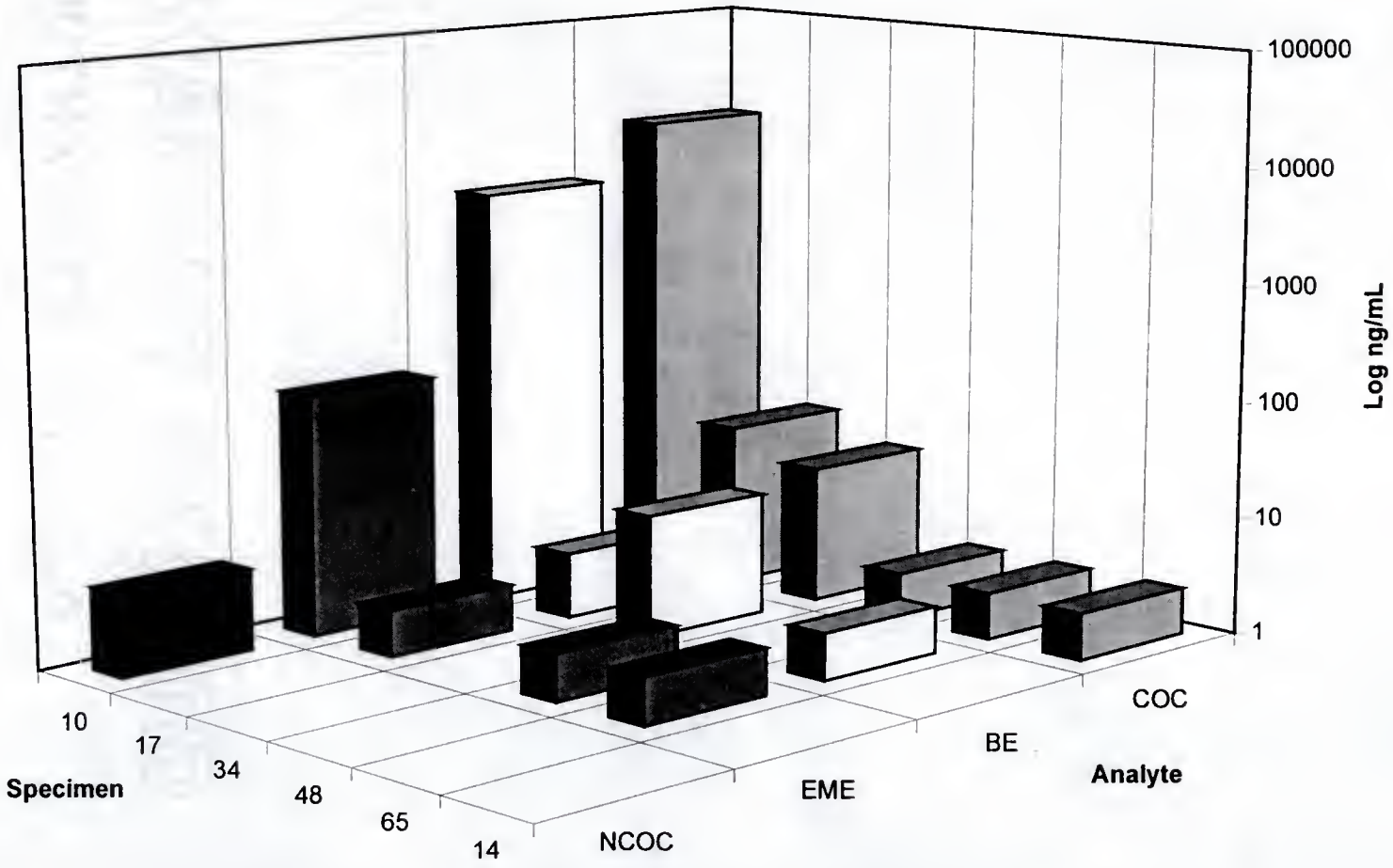


Figure 3-2C. Cocaine analyte results for positive colostrum specimens.

Discussion

The SPE and GC/MS methods described in this study differs from those described by Cone et al. in several significant ways. First, was the employment of the special high flow X-TracT columns to overcome the high viscosity that can occur in amniotic fluid specimens. Second, was the use of the derivatizing reagent MTBSTFA which produced higher molecular weight derivative and improved chromatographic separation than possible with more traditional silyl derivatives. Finally, the volume of elution solvent was increased from 6 to 8 mL. This may have resulted in an improved recovery of EME from the 40% reported by Cone et al. to 76% in amniotic fluid and 90% in umbilical cord tissue reported here.[27] This modified method was found to have excellent sensitivity for the detection of cocaine and its metabolites in amniotic fluid, colostrum, and umbilical cord tissue, and is a satisfactory technique for toxicological testing of these matrices.

The need to investigate new methods of detecting prenatal cocaine exposure is evident by the poor performance of current methods in identifying cocaine exposed infants. Amniotic fluid may prove to have several advantages over maternal or infant urine for the detection of prenatal cocaine exposure. While amniotic fluid is present throughout the gestational period, its composition changes and viscosity decreases as pregnancy progresses due to fetal

urination.[93,99] The result is essentially a pool of fetal urine into which cocaine and metabolites are recirculated by fetal swallowing of the amniotic fluid.[91] There is also evidence that some cocaine metabolites may accumulate in the amniotic compartment [40], and may result in the prolonged presence of cocaine and its metabolites in amniotic fluid, and hence an increased detection window. Finally, amniotic fluid is easier to collect at birth than fetal urine, as urine collection requires the use of uncomfortable and unreliable urine collection bags which irritate infant skin and are easily dislodged.

The appearance of benzoylecgonine, ecgonine methyl ester, and cocaine in amniotic fluid in this study is consistent with previously published data on human amniotic fluid.[96,15,97] Data from previous studies and the current study are summarized in Table 3-IV. In the study by Moore et al., eight amniotic fluid specimens were taken either at amniocentesis or transvaginally at birth from eight women who were known or thought to have abused cocaine during pregnancy. All eight specimens were found to contain benzoylecgonine and/or cocaine by high performance liquid chromatography.[97]

In a continuation of the study by Moore et al., amniotic fluid specimens were taken from 23 subjects with documented cocaine use one day to eight weeks before delivery. Cocaine and/or benzoylecgonine was detected by high performance liquid chromatography in 74% of the specimens. Concentrations of

benzoylecgonine and cocaine ranged from 400 to greater than 5000 ng/mL and trace to 250 ng/mL, respectively.[91]

In the study conducted by Ripple et al., 450 amniotic fluid specimens were obtained during amniocentesis and screened for benzoylecgonine by fluorescence polarization immunoassay (FPIA) in order to evaluate the incidence of cocaine exposure in a low risk population. Five out of the 450 specimens (1%) screened positive by FPIA, and were subsequently confirmed positive for cocaine, benzoylecgonine, and/or ecgonine methyl ester by GC/MS.[15]

In the study by Casanova et al., six amniotic fluid specimens were obtained from admitted cocaine users and analyzed by GC/MS for cocaine and its metabolites. Three of the six specimens (50%) were found to contain benzoylecgonine or ecgonine methyl ester.[96]

In the current study, 53.8% of the amniotic fluid specimens from admitted cocaine users and 10.5% of specimens from those denying use were found to contain cocaine and/or its metabolites. Despite differences in objectives and detection schemes, data from all studies indicate that benzoylecgonine is detected in the greatest amounts, with much smaller amounts of cocaine and ecgonine methyl ester.

The consequences to the fetus of being exposed to amniotic fluid containing cocaine and its metabolites throughout gestation are highly speculative. There is evidence, though, for increased risk to the fetus from this

type of exposure for a variety of reasons. First, the fetus is exposed continually to cocaine and its metabolites transdermally during the first half of pregnancy before keratinization of the skin occurs [93], and the fetus will continue to be exposed during the last half of pregnancy through fetal swallowing of amniotic fluid and reabsorption of these compounds from the gut.[40] In addition, benzoylecgonine and its n-desmethyl metabolite benzoylnorecgonine, have demonstrated powerful vasoconstrictor and convulsant activity, and gestational exposure could result in serious harm to the fetus.[102,103] Finally, since cocaine metabolites apparently accumulate in the fetal compartment due to slow equilibrium with adjacent compartments, the fetus that is continuously and consistently exposed to cocaine during gestation, will be bathed in ever increasing amounts of these hazardous metabolites.[16,40]

The significance of the levels of cocaine and its metabolites found in umbilical cord tissue in this study is unknown at this time, however, certainly analytes found here are representative of, and proportional to, tissue and blood concentrations in the neonate at birth. It is interesting though, that high levels of norcocaine were found in this matrix. This may be an indication of the fetal metabolism of cocaine, which is thought to produce more of the n-desmethyl metabolites.[16]

There is also a paucity of information about the levels of cocaine and its metabolites that can be found in human breast milk or colostrum. Many

pharmaceutical substances have been found to be excreted in breast milk, and in general, the amount that would be delivered through breast feeding to an infant has been found to be modest.[100] It is believed though that cocaine actually partitions selectively into breast milk and colostrum.[101] This is due partly to the lipophilic nature of cocaine and partly to a high blood to breast milk partition coefficient. The result is a concentrating of cocaine in the breast milk and large bolus doses of cocaine being delivered to the neonate during breast feeding.[101]

The levels of cocaine in colostrum found in this study are extremely high with an average of 2000 ng/mL. Since the average amount of breast milk ingested per feeding is 100 mL, the result would be an approximate oral dose of 200 μg cocaine per feeding. Considering the average infant weighs only a few kilograms, this could represent a large dose of cocaine to the infant. This, taken with the possibility of reduced drug metabolism and clearance due to the immature metabolic pathways common in neonates, could result in severe health consequences for the exposed infant.

The short term effects of cocaine intoxication in the breast fed infant have been reported and include tachycardia, tachypnea, hypertension, seizures, sweating and mydriasis.[101, 104] Long term effects possibly include developmental delay and susceptibility to sudden infant death syndrome [55], but there is no substantial evidence linking cocaine exposure from breast feeding to either of

these effects. In fact, there has not been a comprehensive study of how cocaine is partitioned into human breast milk, how long after a cocaine dose it is present, and what long term risks this poses for the neonate.

In conclusion, cocaine and its metabolites are readily detected in amniotic fluid, colostrum and umbilical cord tissue specimens from women and neonates exposed to cocaine. In addition, the current data indicates that amniotic fluid is an appropriate specimen for detecting prenatal cocaine exposure and that colostrum may contain sufficient amounts of cocaine to elicit physiological effects in the neonate after breast feeding. Finally, additional studies are needed to determine the clinical usefulness of umbilical cord tissue and the risks associated with neonatal cocaine exposure by way of breast feeding.

CHAPTER 4
URINE VS. MECONIUM: MORE FUEL FOR THE FIRE

Introduction

The 1980s brought with it a significant increase in cocaine use, and consequently, improvements in the detection and treatment of maternal drug abuse became the goal of many researchers involved in perinatal care. Toward that end, meconium, the neonates first bowel movement, was introduced in the late 1980s as an alternative specimen to maternal or neonatal urine for the detection of gestational drug abuse.[74] While many researchers claim that meconium is a superior specimen for detecting prenatal cocaine exposure, others have been unable to confirm this. This chapter will critically review past meconium research, present the analyses of neonatal urine and meconium undertaken in this current study, and examine whether the results of this study support or dispute the claim that meconium is superior to neonatal urine for detecting gestational cocaine use.

Meconium is the complex intestinal contents of the fetus that collects from approximately week 12 of gestation until birth.[105] It is composed primarily of water (72-80%), with the remainder comprised of bile acids, protein,

mucopolysaccharides, lipids, cholesterol, sterol precursors, epithelial cells, blood group substances, enzymes, and vernix caseosa Amniotic fluid has been proposed as the primary source of water present in meconium, but since knowledge of water absorption in the fetal intestine is limited, this is not known for certain.

Currently, there are two proposed mechanisms by which drugs and/or their metabolites are deposited in meconium. In the first, drugs are incorporated in meconium as the result of fetal swallowing of amniotic fluid containing drug or metabolites that have been excreted in fetal urine.[13,105] In the second, drugs or metabolites are secreted into bile, which is then deposited into the meconium present in the duodenum.[13,40,105]

Meconium is usually passed by the neonate within 5 days following birth. In the 1% of cases in which it is passed before birth, passage rarely occurs before week 34 of gestation.[105] It is precisely because an infant's first bowel movement usually does not occur until after birth that many researchers feel meconium serves as a final depository for drugs to which the fetus has been exposed. The proposed consequence being an improved sensitivity for detection of prenatal drug exposure. In addition, meconium has an advantage as a laboratory specimen due to its ease of collection.

On the other hand, meconium is not a homogenous specimen and it requires substantial pretreatment before analysis.[13,96] In addition, this

heterogeneity makes the preparation of appropriate control materials difficult and in fact, there are no commercial control or proficiency specimens available with which to assess the quality of meconium testing.

Since the first report of meconium analysis in the literature, twelve studies have undertaken a comparison of the sensitivity of meconium for detecting prenatal drug exposure to the traditional specimens of either maternal or neonatal urine. A summary of these studies is presented in Table 4-1.

In 1989, Ostrea et al. reported greater sensitivity for meconium in detecting prenatal cocaine exposure.[74] Subsequently, other researchers have reported similar findings.[16, 56,73,106,107,108] These studies, however, suffered from several deficiencies. First, a more sensitive analytical technique and/or a lower cutoff was commonly used to test the meconium specimens. Moreover, when equally sensitive methods are used, differences between the two specimen types are generally not significant.[56,96,109,110] Second, some of these studies suffer from a small number of test subjects. In fact, 5 of the 12 studies in Table 4-1 have less than 50 subjects. This makes it difficult to determine whether differences between the specimen sensitivities are significant. Finally, several of the studies failed to use an appropriate second analytical method such as GC/MS to confirm specimens that were positive by a screening method.[74,106,107,111] A recent study to determine the false-positive and false-negative rates in meconium drug testing has shown that the use of screen only

results is inappropriate because 43% of positive screens in this study could not be confirmed as positive by GC/MS.[13]

Despite the limitations listed above, meconium has become the most widely accepted alternative specimen to urine for detecting intrauterine drug exposure. Researchers who are active in meconium testing believe meconium to be a superior specimen to urine and claim that: (a) concentrations of drug analytes are greater than in urine[74], (b) meconium provides a longer window for the detection of drug use[13] and (c) meconium assays that monitor for the cocaine metabolite, *m*-hydroxybenzoylecgonine, detect more positives than assays that do not include this analyte.[13,32] Other researchers disagree with these claims and conclude that meconium offers no significant sensitivity advantage over urine and that it does not provide a longer window for the detection of drug use.[96,109] This current study was designed to provide data to address these claims and the analyses described herein will provide information concerning improvements in the detection of gestational cocaine exposure.

Materials and Methods

Subjects

Subjects admitting to cocaine use during their current pregnancy were asked to participate as a target (n=34). Women admitted to labor and delivery

denying cocaine use, were recruited to participate as control subjects (n=47). Participants were confidentially interviewed to assess the amount and timing of drug use during each trimester.

Specimens

All biological specimens were marked with a unique research identification number and the analyst was blinded to drug history. One to five mL of urine was collected in pediatric urine collectors from 75 neonates following parturition. Diapers containing meconium specimens were collected during the first 36 hours of life from 73 neonates. All specimens were stored at -20°C until analysis.

Chemicals

HPLC and GC/MS: Benzoylecgonine (BE), cocaine (COC), ecgonine methyl ester (EME), cocaethylene (CE), norcocaine (NCOC), and trideuterated analogs for BE, EME, COC, and CE were purchased from Radian Corporation (Austin, TX). Benzoylnorecgonine (BNE) was purched from RBI. Ecgonine ethyl ester (EEE) and *m*-hydroxybenzoylecgonine (MOHBE) were a generous gift from Edward Cone, Ph.D. Methyl-(tert-butyldimethylsilyl)-trifluoracetamide (MTBSTFA) was purchased from Pierce Chemical Company (Rockford, IL). All

other reagents were of reagent grade and were purchased from Fisher Scientific (Orlando, FL).

Immunoassay: EMIT II Cocaine Metabolite Assay (Syva # 9H019, Syva Co, San Jose, CA), CEDIA Cocaine Assay (Catalog # 1404230, Boehringer Mannheim Corp., Indianapolis, IN), Negative control (QA Services, Inc., Augusta, GA), Positive control (QA Services, Inc., Augusta, GA), Intermediate Control (QA Services, Inc., Augusta, GA), 4-Drug Cutoff Calibrator (Catalog # 946358), and Negative Calibrator (Catalog # 946377). All calibrators and controls were purchased from Boehringer Mannheim Corp., except where noted otherwise.

Standard preparation

GC/MS: Calibration standards were prepared by fortifying the appropriate blank specimen type with aliquots of standard solutions to form a calibration curve in the range of 25-750 ng/mL or ng/g. Control specimens prepared at 0, 200, and 600 ng/mL, or ng/g, for all analytes were included in each analytical run. In addition, a 500 ng/mL cocaine standard was included in each run to monitor hydrolysis of COC to BE and/or EME during extraction and subsequent analysis. All standards and controls were prepared immediately prior to extraction.

HPLC: Calibration standards were prepared by fortifying the appropriate blank specimen type with aliquots of standard solutions to form a calibration curve in the range of 62.5-4000 ng/mL or ng/g. Control specimens prepared at 0, 75, and 3200 ng/mL, or ng/g, for all analytes were included in each analytical run. All standards and controls were prepared immediately prior to extraction.

Extraction of Biological Matrices

The solid phase extraction (SPE) of cocaine and its metabolites was adapted from the procedure previously described by Cone et al.[16]

Meconium: Approximately 1 g of meconium was weighed into a culture tube and bupivacaine (1 μg) was added as an internal standard. The weighed meconium was vortexed with 3 mL of methanol for up to 2 min. to form a uniform homogenate. Homogenates were centrifuged for 10 min at 1000 x g, and the supernatants were decanted into clean culture tubes and dried under a gentle nitrogen stream at room temperature.

The dried extracts were reconstituted in 3 mL of phosphate buffer (0.025M, pH 4) and 250 μL was diverted for immunoassay, while the remainder was applied to CleanScreen™ SPE extraction columns (ZSDAU020; United Chemical Technologies, Horsham, PA) that had previously been conditioned with elution solvent (1 x 1 mL), methanol (1 x 3 mL), deionized water (1 x 3 mL), and 0.025M (pH 4) phosphate buffer (1 x 2 mL). The specimens were followed

with a wash of deionized water (1 x 2 mL) and 0.1M HCl (1 x 2 mL). The columns were then air dried at full vacuum for 2 min. A methanol wash (1 x 6 mL) and a second 2 min. drying step completed the washes. The analytes were then collected in culture tubes by eluting with 8 mL of the elution solvent (methylene chloride: isopropanol: concentrated aqueous ammonium hydroxide, 80:20:2 by vol). The extracts were evaporated to dryness at 40°C under a gentle nitrogen stream. Once dry, the extracts were reconstituted in 50 μL of acetonitrile and split for analysis by both HPLC and GC/MS. Twenty microliters was diluted with 180 μL of mobile phase for HPLC analysis and the remainder was derivatized for GC/MS analysis by adding MTBSTFA (30 μL) and heating at 90°C for 60 min.

Urine: To 1 mL of urine, bupivacaine (1.0 μg) was added as an internal standard, followed by 3 mL of phosphate buffer (0.025M, pH 4). The specimens were then applied to the SPE columns for extraction and analysis in the same manner as described above.

Screening Analyses

All EMIT and CEDIA immunoassays for the cocaine metabolite, benzoylecgonine, were performed in parallel on a Hitachi 717 Automatic Analyzer (Boehringer Mannheim Corp., Indianapolis, IN) according to the manufacturers specifications. All reagents and controls were prepared

according to the manufacturer as directed in the product inserts.[113,114]

The negative calibrator was assigned a value of zero. The 4-Drug cutoff calibrator (300 ng/mL) was used as the reference in distinguishing between positive and negative samples and was assigned a value of 100%. Any specimens reading below 100% are considered negative, any specimen reading ≥100% was considered positive. The positive control was targeted at a concentration 25% above the 300 ng/mL cutoff and the intermediate control was targeted at a concentration 25% below. For a run to be considered in control, the negative control should read below 10%, the 25% below cutoff control must read below 100%, and the 25% above cutoff control must read above 100%. Any run found not to be in control was repeated.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed using a Waters 501 pump to deliver mobile phase at 1.5 mL/min to an Alltech (Deerfield, IL) Lichrosorb RP-18 10μm column (25cm X 4.6 mm i.d.). A Waters C -18 Guard Pak precolumn was used to protect the analytical column. The detector was a Waters 996 Photodiode Array, monitored at 230, 255 and 275 nm, and was controlled by Waters Millennium PDA software on a Dell computer. The mobile phase consisted of 0.025 m potassium phosphate buffer: acetonitrile: butylamine (81:18:1). The pH was adjusted to 2.9 with concentrated orthophosphoric acid, filtered and degassed

prior to use. The samples were injected using a Waters 717 Plus WISP autosampler with a 96 sample capacity. The sample size injected was 50 μL. Quantitative analysis was achieved by the ratio of analyte peak areas to internal standard versus a standard curve of extracted standards.

Gas Chromatography/ Mass Spectrometry (GC/MS)

GC/MS analyses were performed with a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a Hewlett-Packard 7673A automated liquid sampler and interfaced with a Hewlett-Packard 5972A mass selective detector. The GC and detector were controlled by a Hewlett-Packard data system. The GC column was a HP-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness). Helium was the carrier gas, programmed at a constant flow rate of 1 mL/min. The injection port was fitted with a 2-mm silanized glass liner. The injection port temperature was 275°C, and the detector temperature was 290°C.

The initial oven temperature of 90°C was maintained for 1 min, programmed to 220° C at 30°C /min and held for 0.5 min, then programmed to 330°C at 20°C /min and maintained for 1 min. The total run time was 12.33 min. The GC/MS was operated in the selected ion monitoring (SIM) mode with a dwell time of 20 ms/ion and quantitation was based upon ion peak area ratios of analyte to internal standard.

Results

The calibration curves for each analyte were found to be linear over the calibration range by use of the standard error of the estimate statistic (Appendix I, Curves I-8 to I-22 and Appendix II, Curves II-1 to II-8). Limit of detection (LOD) was defined as the concentration corresponding to a signal to noise ratio of 3. The limit of quantitation (LOQ) was determined by the analysis of a series of decreasing standards and defined as the lowest standard that did not deviate from the target concentration by more than 20%. Recovery and precision were measured at two concentrations. Level one at 200 ng/mL or ng/g, and level two at 600 ng/mL or ng/g, for GC/MS and level one at 350 ng/mL or ng/g and level two at 3200 ng/mL or ng/g for HPLC. The LOD, LOQ, recovery, and precision are presented in Tables 4-2 for GC/MS and Tables 4-3 for HPLC.

Figure 4-1,A-D illustrates a SIM chromatogram of an unextracted standard (A), a 600 ng/mL extracted standard (B), a negative quality control specimen (C) and a positive urine specimen obtained from a target subject (D). The target specimen was found to contain benzoylecgonine (18000 ng/mL), ecgonine methyl ester (5714 ng/mL), norcocaine (102 ng/mL), cocaine (585 ng/mL) and *m*-hydroxybenzoylecgonine (360 ng/mL).

Figure 4-2,A-C illustrates a HPLC chromatogram of a 3200 ng/g meconium standard (A), a negative quality control specimen (B) and a positive

meconium specimen obtained from a target subject (C). The target specimen was found to contain benzoylecgonine (1536 ng/g), cocaine (282 ng/g), and a trace amount of norcocaine.

The immunoassay results for each specimen and type are presented in Figure 4-3. On average, responses for the same specimen were 70% higher using the EMIT assay for both urine and meconium. The distribution of the specimen responses, as measured by the EMIT assay, from < 25% (corresponding to 75 ng/mL) to >100% (corresponding to 300 ng/mL) and the percent in each category that were confirmed positive are presented in Table 4-4. Among specimens testing positive at the 300 ng/mL cutoff, 100% were confirmed positive by GC/MS for both urine and meconium. However, this only identified 22.6% and 25.8% of urine and meconium target specimens, respectively and none of the control specimens that were subsequently determined to be positive by HPLC or GC/MS (Table 4-5).

The percent of specimens testing positive by immunoassay, HPLC and GC/MS by subject type are presented in Table 4-5. The immunoassay results were previously discussed. An approximately equal number of urine and meconium target specimens tested positive by HPLC and in both cases, approximately the same percent were confirmed by GC/MS. Among controls, more urine specimens tested positive than meconium specimens by HPLC, but a smaller percentage of the urine specimens were confirmed by GC/MS. This

resulted in an approximately equal percentage of positive urine and meconium control specimens. The qualitative results and number of days since the last reported drug use for each target subject are presented in Table 4-6. There was not a significant difference (p=0.75, McNemar's Test, Exact Method) between the results of paired meconium and urine specimens.[89]

The analyte concentrations for each positive urine (n=19) and meconium (n=20) specimens are presented in Table 4-7 and 4-8, respectively. In general, benzoylecgonine was detected in the highest concentrations in both urine and meconium. In meconium, benzoylecgonine was followed by cocaine, ecgonine methyl ester, norcocaine, *m*-hydroxybenzoylecgonine, ecgonine ethyl ester, and cocaethylene. In urine, benzoylecgonine was followed by ecgonine methyl ester, *m*-hydroxybenzoylecgonine, cocaine, and norcocaine. Ecgonine ethyl ester and cocaethylene were not detected in any urine specimen. Analyte totals for paired urine and meconium positive specimens are presented in Figure 4-4. Statistical analysis revealed that analyte concentrations in urine specimens are significantly higher than concentrations in meconium specimens (p=0.03, paired t-test).[89] The percent of positives by specimen type and analyte are presented in Table 4-9. Benzoylecgonine was the most common analyte detected, present in 100% of positive urine specimens and 85% of positive meconium specimens. The frequency of the other analytes detected was variable between the two specimen types.

Table 4-1. Review of the meconium comparison studies.

Investigator	Subjects (n)	Meconium Analytical Method(s)	Cutoff (ng/g)	% positive	Urine Analytical Method	Cutoff (ng/mL)	% positive	Comments
Ostrea et al., 1989	20	RIA	15	80	FPIA	300	37	
Maynard et al., 1991	26	RIA	300	61.5	EMIT, GC/MS	300	50	p=2
Callahan et al., 1992	59	FPIA, GC/MS	150	52, 74	EMIT	300	38	p=0.06, p=0.015
Ostrea et al., 1992	335	RIA	15	77	?	?	46	
Dahlem et al., 1992	20	RIA	?	30	RIA	?	20	
Dusick et al., 1993	232	HPLC, GC/MS	?	37%	EMIT	?	25.9	
Browne et al., 1994	106	HPLC	?	19.8	EMIT	?	7.5	
DiGregorio et al., 1994	40	GC/MS	40	70	GC/MS	20	*50	*maternal urine
Moriya et al, 1994	50	EMIT, GC/MS	100, 75	16	EMIT, GC/MS	300, 10	18	
Casanova et al., 1994	30	GC/MS	5	66.7	GC/MS	5	66.7	
Ryan et al., 1994	100	FPIA, GC/MS	60	28	FPIA, GC/MS	50	21	p=0.016
Wingert et al., 1994	345	EMIT, GC/MS	?	11.9	EMIT, GC/MS	?	12	
Lewis et al., 1995	54	FPIA, GC/MS	100	25.9	EMIT	300	9.3	
Bibb et al., 1995	29	EMIT, GC/MS	150	4.5	EMIT GC/MS	300	2.7	

Table 4-2A. Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery, and Precision of Cocaine Analytes in Neonatal Urine by GC/MS.*

Analyte	LOD*	LOQ*	Recovery (%)		Within Run Precision (%)		Between Run Precision (%)	
			Level 1**	Level 2**	Level 1	Level 2	Level 1	Level 2
EME	2.5	25	100	108	3.3	2.3	12.8	6.3
EEE	25	50	84	110	5.5	2.1	19.3	14.6
NCOC	2.5	25	115	126	3.6	2.7	20.6	7.2
COC	1.25	10	116	105	2.5	2.9	8.1	8.0
CE	5	10	118	104	2.7	2.4	11.6	6.4
BE	2.5	5	120	106	5.0	2.0	9.9	7.8
BNE	50	50	31	46	8.6	4.6	54.8	40.1
MOHBE	5	10	104	117	7.6	7.1	19.7	10.5

Table 4-3A. Limit of Detection (LOD), Limit of Quantitation (LOQ), and Precision of Cocaine Analytes in Neonatal Urine by HPLC*

Analyte	LOD*	LOQ*	***Within Run Precision (%)		Between Run Precision (%)	
			Level 1	Level 2	Level 1	Level 2
NCOC	10	25	8.0	3.4	9.4	10.0
COC	10	25	10.2	4.1	4.4	4.7
CE	10	50	3.5	3.8	14.9	11.3
BE	10	25	1.2	4.2	10.3	4.3

*Concentrations noted in ng/mL
** Level 1 is 100 ng/mL and Level 2 is 500 ng/mL
***Level 1 is 350 ng/mL and Level 2 is 3200 ng/mL.
Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 4-2B. Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery, and Precision of Cocaine Analytes in Meconium by GC/MS*

Analyte	LOD*	LOQ*	Recovery (%)		Within Run Precision (%)		Between Run Precision (%)	
			Level 1**	Level 2**	Level 1	Level 2	Level 1	Level 2
EME	5	10	95	82	7.8	4.7	19.7	9.1
EEE	25	50	99	80	9.7	9.9	37.2	22.9
NCOC	5	25	108	92	8.2	2.8	20.6	11.9
COC	5	10	93	90	2.9	3.4	7.5	7.9
CE	5	10	86	90	2.2	2.9	15.3	9.4
BE	2.5	10	97	94	5.5	2.6	12.2	9.0
BNE	25	50	36	49	9.1	14.3	76.5	27.9
MOHBE	10	25	69	99	5.5	6.0	16.5	10.4

Table 4-3B. Limit of Detection (LOD), Limit of Quantitation (LOQ), and Precision of Cocaine Analytes in Meconium by HPLC*

Analyte	LOD*	LOQ*	***Within Run Precision (%)		Between Run Precision (%)	
			Level 1	Level 2	Level 1	Level 2
NCOC	25	50	7.5	4.4	18.5	13.8
COC	25	50	6.2	5.9	11.4	8.4
CE	25	50	3.6	4.1	19	4.7
BE	25	50	7.2	2.8	21.1	6.1

*Concentrations noted in ng/g
** Level 1 is 100 ng/g and Level 2 is 500 ng/g.
***Level 1 is 350 ng/g and Level 2 is 3200 ng/g.
Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 4-4. Immunoassay response and percent positives by specimen type.

Response* (ng/mL)**	Urine n (%)***	Confirmed n (%)	Meconium n (%)	Confirmed n (%)
>100 (>300)	7 (9.3)	7 (100)	8 (10.9)	8 (100)
75-100 (225-300)	2 (2.7)	2 (100)	2 (2.7)	2 (100)
50-75 (150-225)	2 (2.7)	2 (100)	3 (4.1)	2 (66.7)
25-50 (75-150)	3 (4.0)	2 (66.7)	5 (6.8)	2 (40.0)
<25 (<75)	61 (81.3)	6 (9.8)	55 (75.3)	6 (8.2)

*Defined as the % response of the cutoff calibrator (300 ng/mL) which is assigned a value of 100%.
**Number in parentheses represents the approximate benzoylecgonine concentration in ng/mL corresponding to the response value.
*** Percent of all specimens having this response.

Table 4-5. Positives by GC/MS, HPLC, Immunoassay, and Subject Type.

Method	Urine Positives n (%)	Confirmed (%)	Meconium Positives n (%)	Confirmed (%)
Immunoassay*				
Control	0	NA***	0	NA
Target	7 (22.6)	100	8 (25.0)	100
All	7 (9.3)	100	8 (10.8)	100
HPLC**				
Control	16 (36.4)	18.8	7 (16.7)	28.6
Target	18 (58)	66.7	19 (59.4)	78.9
All	34 (45)	44.1	26 (35.1)	65.4
GC/MS**				
Control	4 (9.1)	NA	4 (9.5)	NA
Target	15 (48.4)	NA	16 (50.0)	NA
All	19 (25.3)	NA	20 (27.0)	NA

* 300 ng/mL benzoylecgonine cutoff.
** cutoff concentration is equal to the Limit of Detection.
*** NA= not applicable.

Table 4-6. Qualitative results of pairs and number of days since last cocaine use from maternal interview.

ID	Urine	Meconium	Days Since Last Use
2	Negative	Positive	49
4	Negative	Negative	180
5	Negative	Negative	236
8	Positive	Positive	22
10	Positive	Positive	4
11	Negative	Negative	98
13	Positive	Positive	0
14	Negative	Negative	203
17	Positive	Positive	0
20	Negative	Negative	147
23	Positive	Positive	2
25	Positive	Positive	0
27	Negative	Negative	12
30	Negative	Negative	118
34	Positive	Positive	92
41	Positive	Positive	3
45	Negative	Negative	202
47	Negative	Negative	52
48	Negative	Negative	14
53	Positive	Positive	3
54	Negative	Positive	102
56	Positive	Positive	1
60	Positive	Negative	3
61	Positive	Positive	1
68	Negative	Negative	3
76	Positive	Positive	7
78	Negative	Negative	180
85	Negative	Negative	62

Table 4-7. Results and enrollment status of positive neonatal urine specimens.

ID	Enrollment Status	EME	EEE	NCOC	COC	CE	BE	MOHBE
8	T	27			TR		50	36
10	T	159					173	38
13	T	TR					1522	31
17	T	5714		102	585		18000	360
18	C						TR	
19	C						TR	
23	T	78					634	31
25	T	231			TR		4859	56
34	T	TR					TR	TR
41	T	412			TR		17037	285
52	T	55					TR	273
53	T	TR					257	
56	T	551			34		29804	858
60	T						97	TR
61	T	443			87		12956	550
65	T			TR	TR		91	
71	C				TR		TR	TR
75	C						TR	
76	T	229			43		3426	TR
Average		789		102	187.25		6838.92	251
Std. Dev,		1739			266.18		9576.69	278

Abbreviations are as follows: C=control subject, T=target subject, TR=trace, Std. Dev. = standard deviation, ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 4-8. Results and enrollment status of positive meconium specimens.

ID	Enrollment Status	EME	EEE	NCOC	COC	CE	BE	MOHBE
2	T							82
8	T						TR	
10	T	320	233	TR	52		111	465
13	T	739		154	1225		644	TR
17	T	407		53	282	34	1536	226
23	T	837			816		594	73
25	T	266			250		723	41
34	T	25			21		96	TR
35	C						TR	
41	T			922	4774		2288	
42	T	510		TR	442		648	167
53	T	622			168		221	105
54	T	92						
56	T	131	85	TR	176	30	262	TR
58	C				84		122	
61	T	2532		155	1994		3711	94
62	C	112			112		156	
65	T	TR						
73	C	TR		TR	TR	TR	TR	TR
76	T	21			TR		350	TR
Average		508	159	321	799	32	818	156
Std Dev		665	104	403	1324	2.83	1037	137

Abbreviations are as follows: C=control subject, T=target subject, TR=trace, Std. Dev. = standard deviation, ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 4-9. Percent of specimens testing positive by specimen type and analyte.

Specimen	EME	EEE	NCOC	COC	CE	BE	MOHBE
Urine							
Positives (%)	68	0	10.5	47	0	100	74
All (%)	17	0	2.7	12	0	25	18.7
Meconium							
Positives (%)	75	10	40	75	15	85	65
All (%)	20.5	2.7	11	20.5	4.1	23	17.3

Figure 4-1A. Total ion chromatogram from an Unextracted Standard. Internal Standard: bupivacaine (BUPI) Analytes: Ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), *m*-hydroxybenzoylecgonine (MOHBE) are readily detected in the standard.

File : C:\HPCHEM\1\DATA\WINECKER\05NOV95A.02P\001SP001.D
Operator : R. WINECKER
Acquired : 5 Nov 95 2:44 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: UNEXTRACTED STANDARD
Misc Info :
Vial Number: 1

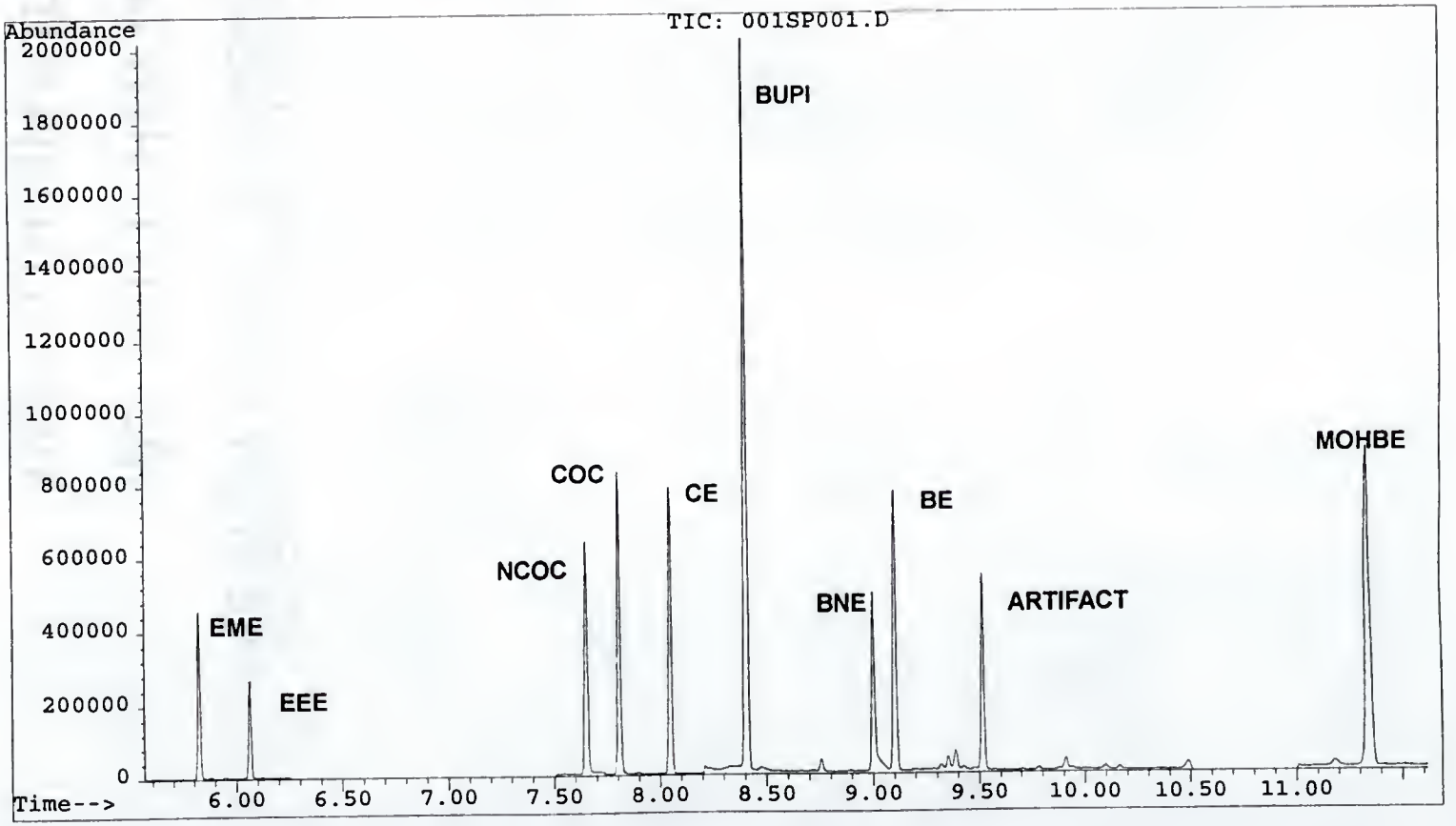


Figure 4-1B. Total ion chromatogram of an extract from a 600 ng/mL quality control extracted standard. Internal Standard: Bupivacaine (BUPI) Analytes: Ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), *m*-hydroxybenzoylecgonine (MOHBE) are readily detected in the standard.

File : C:\HPCHEM\1\DATA\WINECKER\05NOV95A.02P\026SP026.D
Operator : R. WINECKER
Acquired : 5 Nov 95 9:29 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: 600 NG/ML QC
Misc Info :
Vial Number: 26

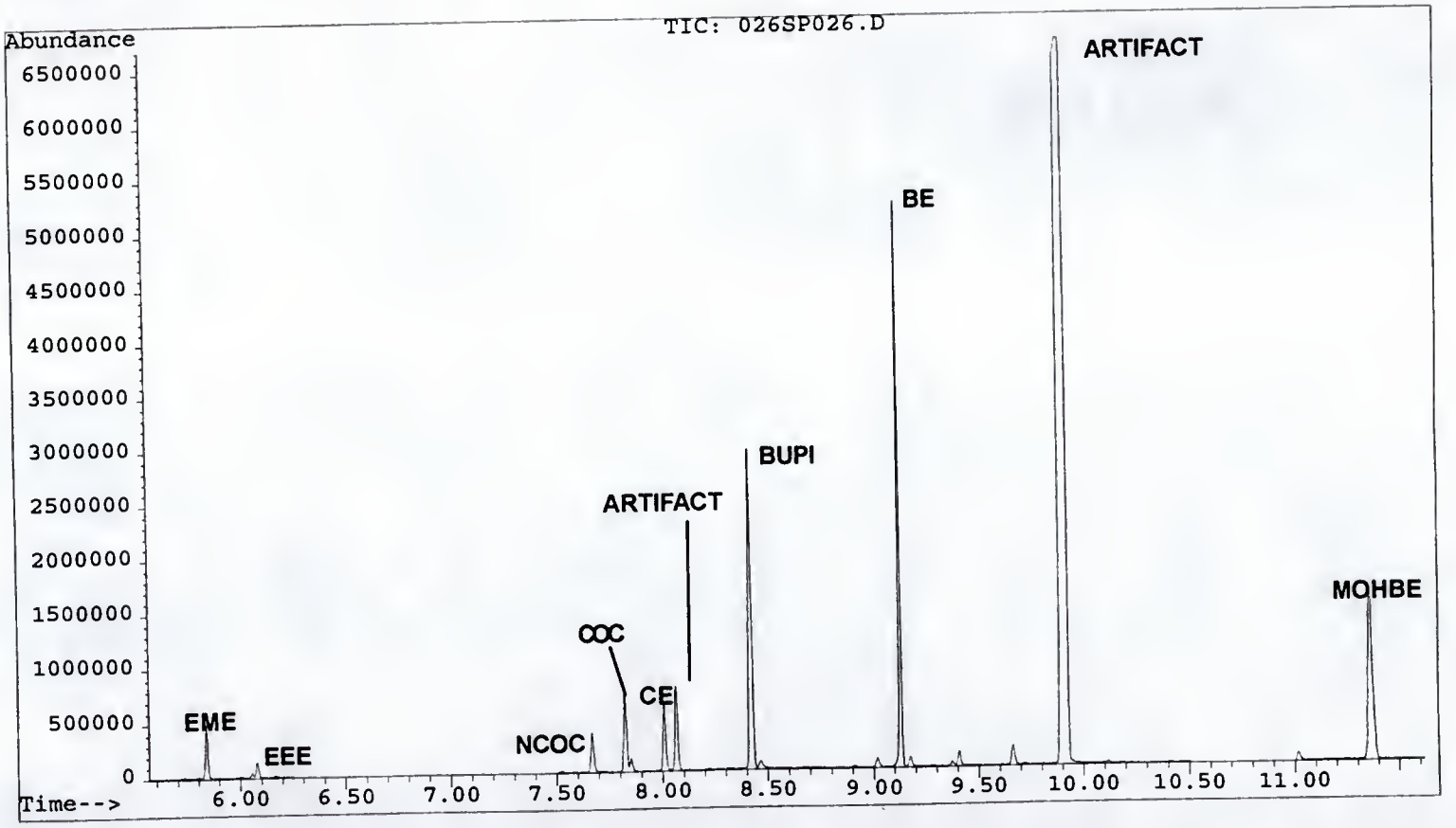


Figure 4-1C. Total ion chromatogram of an extract from a negative quality control standard. Internal Standard: Bupivacaine (BUPI).

File : C:\HPCHEM\1\DATA\WINECKER\05NOV95A.02P\023SP023.D
Operator : R. WINECKER
Acquired : 5 Nov 95 8:40 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: NEG
Misc Info :
Vial Number: 23

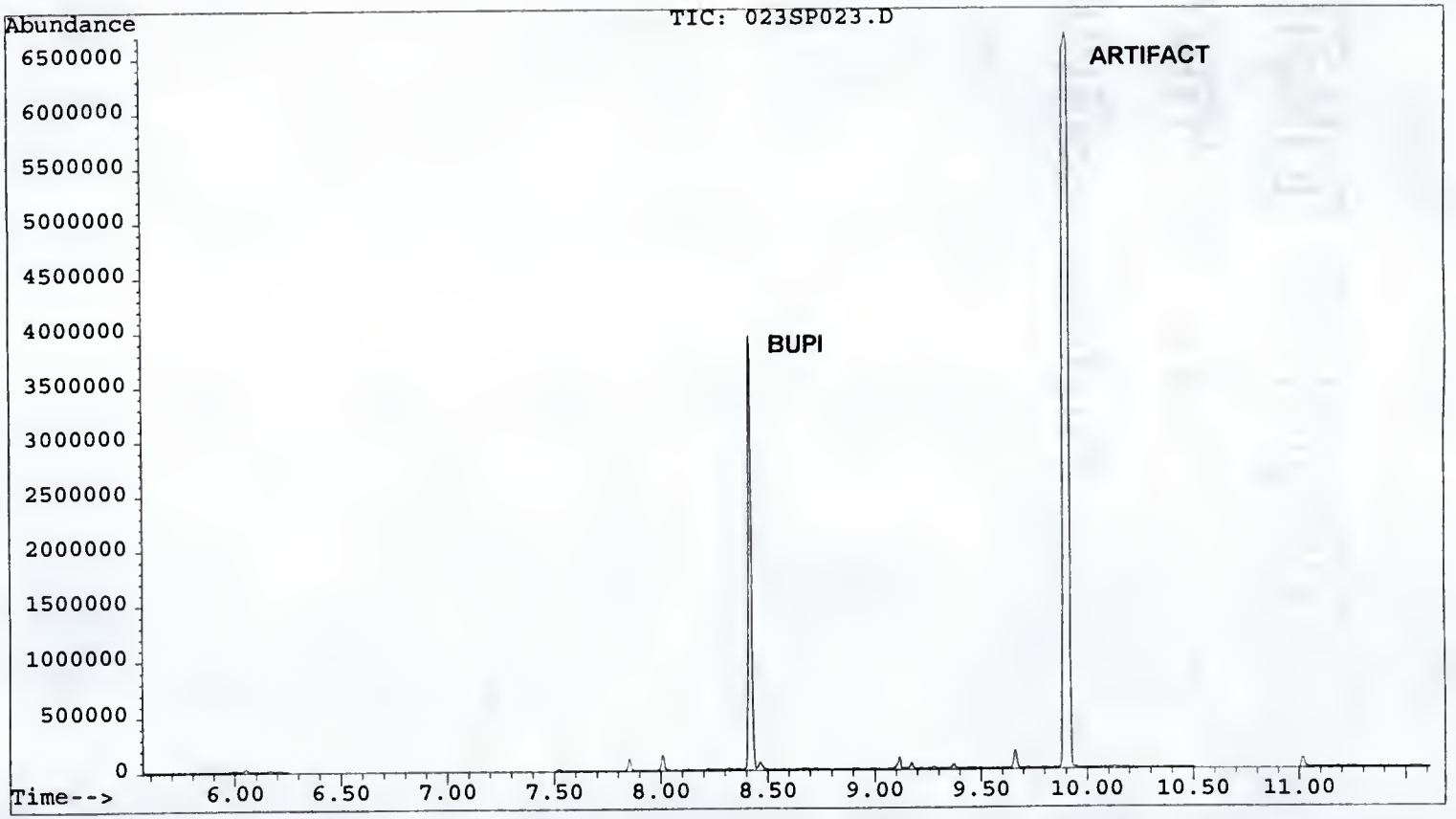


Figure 4-1D. Total ion chromatogram of an extract from a positive urine specimen obtained from a target subject. Internal Standard: Bupivacaine (BUPI) Analytes: The target specimen was found to contain benzoylecgonine (18000 ng/mL), ecgonine methyl ester (5714 ng/mL), norcocaine (102 ng/mL), cocaine (585 ng/mL), and *m*-hydroxybenzoylecgonine (360 ng/mL).

File : C:\HPCHEM\1\DATA\WINECKER\05NOV95A.02P\008SP008.D
Operator : R. WINECKER
Acquired : 5 Nov 95 4:38 pm using AcqMethod COCMB.M
Instrument : 5972 - MS
Sample Name: CS1017 * 2
Misc Info :
Vial Number: 8

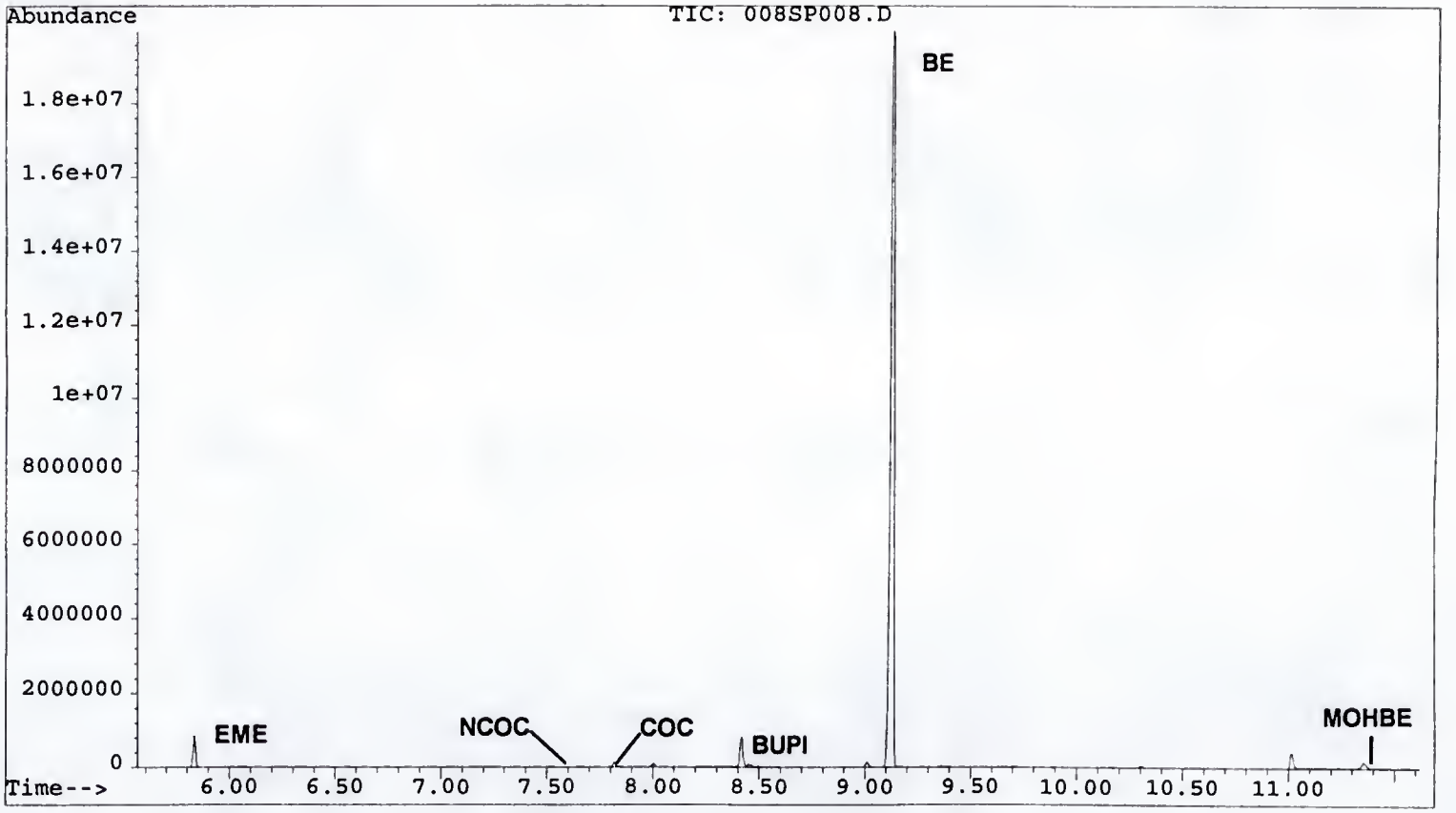


Figure 4-2A. HPLC chromatogram of an extract from a 3200 ng/g extracted standard. Internal Standard: Bupivacaine (BUPI) Analytes: norcocaine (NCOC), cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), are readily detected in the standard.

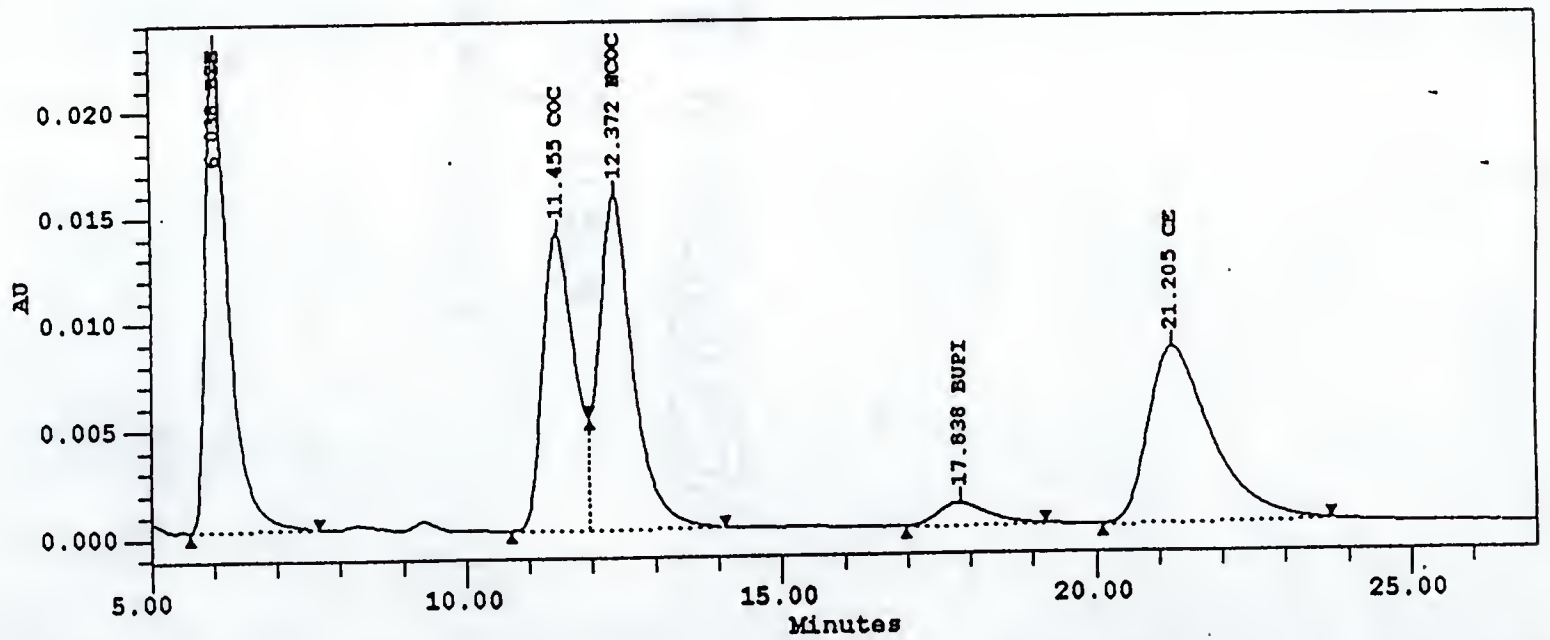


Figure 4-2B. HPLC chromatogram of an extract from a negative quality control standard. Internal Standard: Bupivacaine (BUPI).

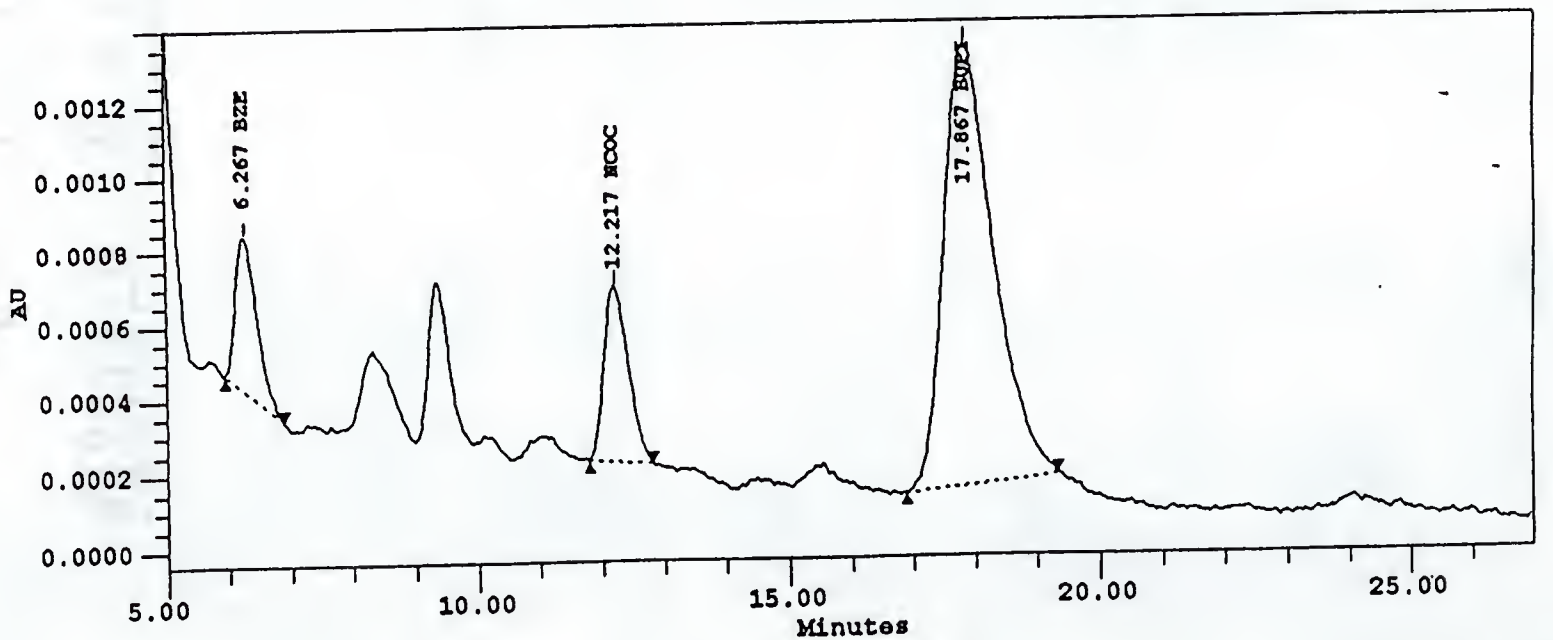
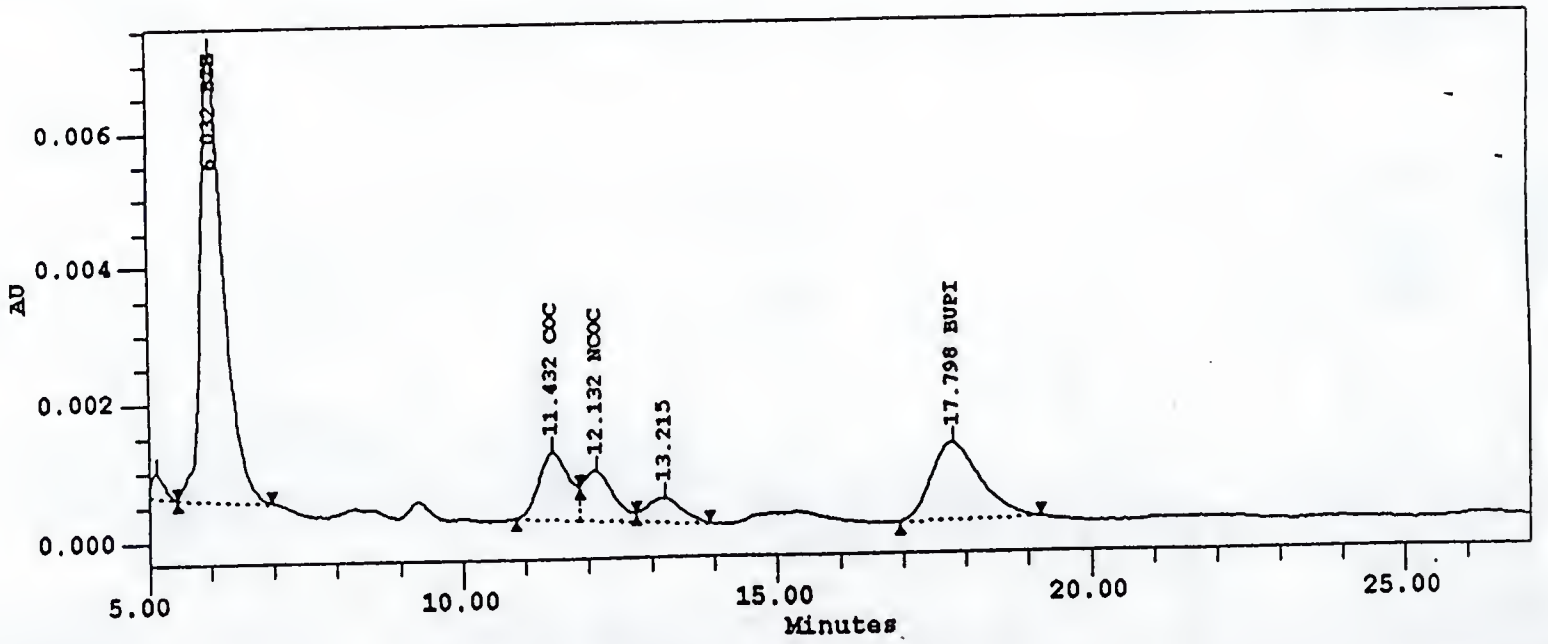


Figure 4-2C. HPLC chromatogram of an extract from a positive meconium specimen obtained from a target subject. Internal Standard: Bupivacaine (BUPI) Analytes: The target specimen was found to contain benzoylecgonine (1536 ng/g), norcocaine (trace), and cocaine (282 ng/g).



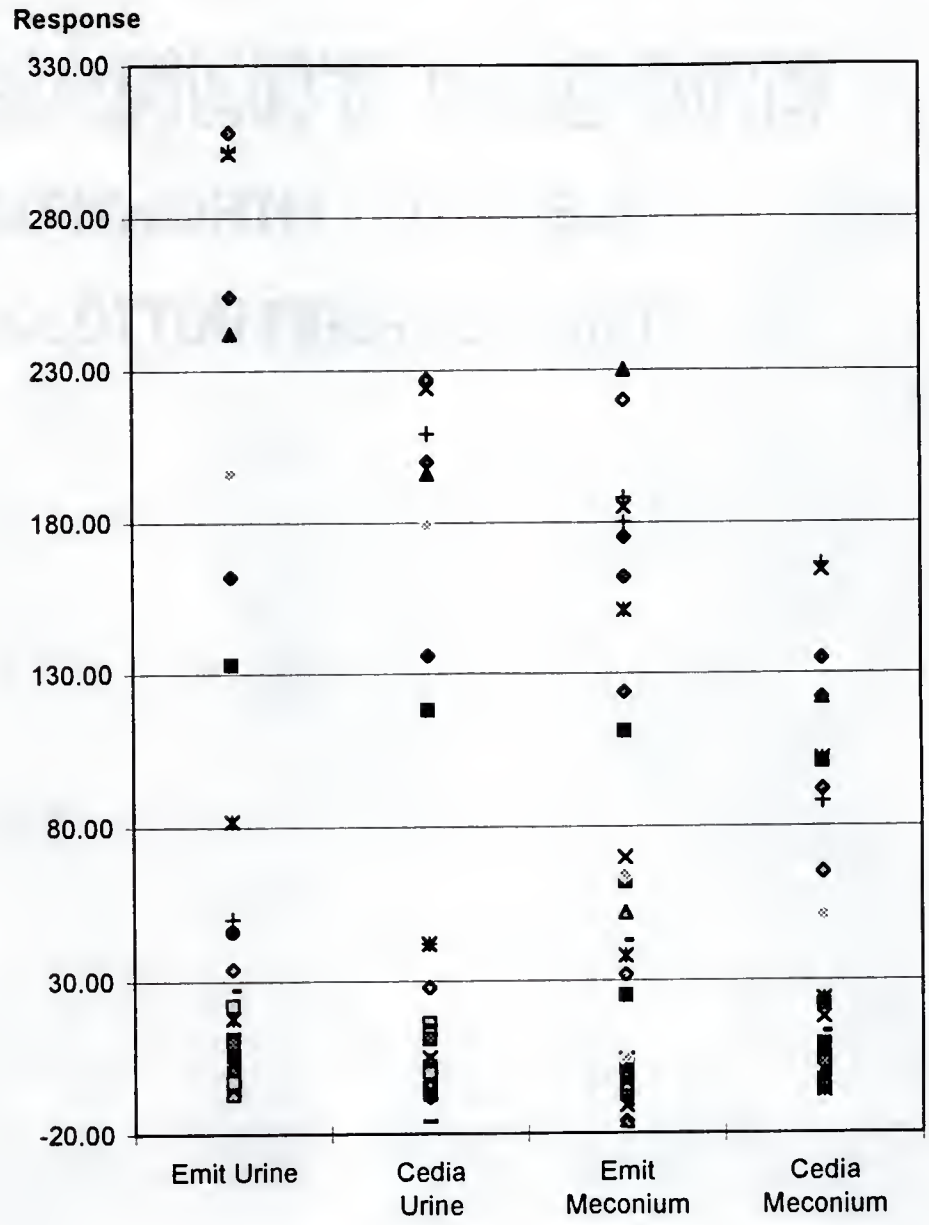


Figure 4-3. EMIT and CEDIA Immunoassay Results of All Screened Specimens by Specimen Type
(Note: Response of 100 is equal to a benzoylecgonine concentration of 300 ng/mL)

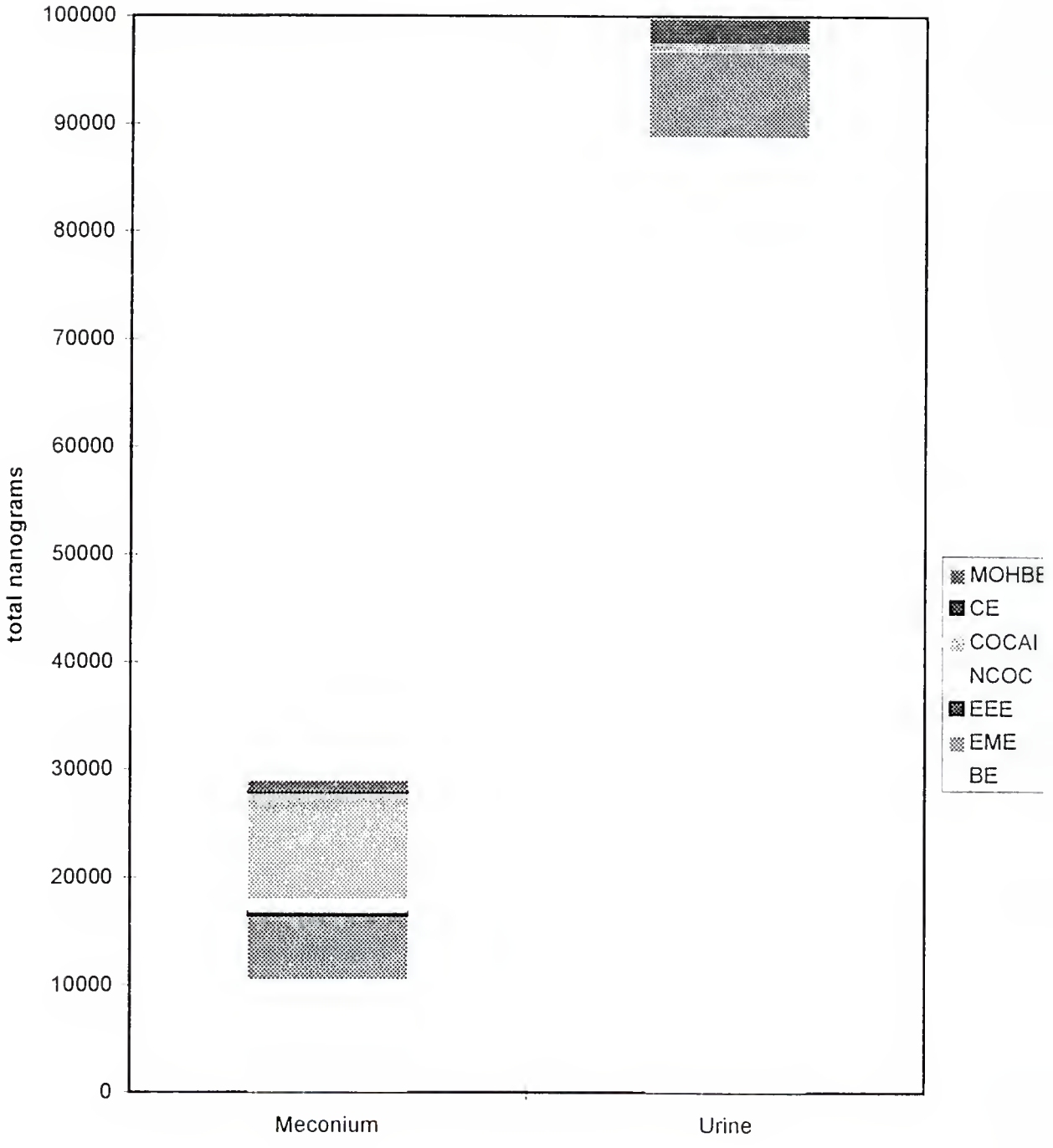


Figure 4-4. Analyte totals for parired urine and meconium specimens.

Discussion

The SPE, HPLC, and GC/MS methods described here were found to have excellent sensitivity for the detection of cocaine and most of its metabolites in meconium and neonatal urine. Therefore, these methods are satisfactory techniques for the toxicological testing of these specimens. The CVs for several analytes were at the high end of the acceptable range. This was probably due to the low intensity of the quantitation ion, poor recovery, and the use of a non-isotopic internal standard. The recovery and precision for the cocaine metabolite benzoylnorecgonine were unacceptably low and the extraction of this analyte was found to be unreliable. Attempts to improve the extraction for benzoylnorecgonine were unsuccessful and resulted in decreased sensitivity for the other analytes. Therefore, the specimen results do not include data for, or discussion of, benzoylnorecgonine.

The majority of commercially available immunoassay kits include a 300 ng/mL benzoylecgonine cutoff calibrator, required for workplace drug testing by the Substance Abuse and Mental Health Services Administration (SAMHSA), for use in distinguishing between positive and negative specimens.[113,114] This cutoff level is designed primarily for use in workplace drug testing programs and is an administratively established value that has little to do with the ability of the immunoassay to determine the presence of benzoylecgonine in specimens at

concentrations below 300 ng/mL. Moreover, it is certainly possible to lower the cutoff concentration, as the published LODs for the CEDIA and EMIT are 7.3 ng/mL and 4.7 ng/mL, respectively.[112]

It is clear from the immunoassay data in this current study, that the use of the 300 ng/mL administrative cutoff for benzoylecgonine is not appropriate for the identification of cocaine exposed neonates. Only 7 of 19 positive urine specimens (36.8%) and 8 of 20 positive meconium specimens (40%) screened above the 300 ng/mL cutoff. By lowering the cutoff to 75 ng/mL (Table 4-4), 13 of 19 (68%) and 14 of 20 (70%) of positive urine and meconium specimens, respectively are identified. There were 6 false negatives and 1 false positive for urine and 6 false negatives and 3 false positives for meconium at this level. The fact that the instrument was calibrated at the 300 ng/mL cutoff level means that the assay was optimized for determining positives from negatives at this level Further improvements in sensitivity and specificity might have been possible had the assay been calibrated at the 75 ng/mL level.

The antibody used in these immunoassays is selective for benzoylecgonine, the principal hydrolysis product of cocaine in adult urine. In the EMIT assay, the antibody exhibits a less than 1% reactivity towards cocaine or other metabolites such as norcocaine or cocaethylene.[113] On the other hand, CEDIA reagents exhibit a 60% cross reactivity for cocaine and cocaethylene with the benzoylecgonine antibody.[114] In positive specimens, 47% of urine and 75%

of meconium specimens contained cocaine (Table 4-9). In light of this, the higher response observed for the EMIT assay, seen in Figure 4-3, is at first, counterintuitive. The lower sensitivity exhibited by the CEDIA assay may be explained by differences in the antibodies used in CEDIA and EMIT assays. The CEDIA antibody exhibits a significant amount of cross reactivity with cocaine analytes other than benzoylecgonine, which means that the antigen-antibody complex is probably not as stable as the antigen-antibody complex in the EMIT assay. This lower stability would result in a lower signal for the CEDIA assay. In addition, the CEDIA and EMIT assays employ different enzymes to generate a signal and there may be some difference in the amounts of product and consequently, signal generated in response to the presence of analyte. This is not the first experiment to report a difference between the EMIT and CEDIA benzoylecgonine assays. Wu et al. reported CEDIA to have lower signal than EMIT when the assays were run in parallel.[112]

The HPLC method exhibits better sensitivity than immunoassay for the screening of specimens. Fifteen of 19 (79%) urine specimens and 17 of 20 (85%) meconium specimens were identified by HPLC (Table 4-5). Specifically, the urine specimens that were not detected by HPLC were 52, 60, 65, and 71 (Table 4-7), and were also the specimens containing the lowest amounts of analytes. Likewise, the meconium specimens not identified were 35, 65, and 73 (Table 4-8).

The SPE procedure exhibited excellent recovery of the analytes under investigation. The downside of this recovery, though, may have been dirtier extracts containing other endogenous substances that would absorb UV light and decrease the specificity of the HPLC assay. The number of false positives by HPLC reinforces the need to pair screening methods with more specific methods, such as GC/MS, especially when the analysis involves the cleanup and extraction of complex specimens such as urine or meconium.

The GC/MS analysis of paired meconium and neonatal urine specimens did not reveal meconium to be a more sensitive specimen for the detection of prenatal cocaine exposure. This is consistent with work done by Casanova et al., in which 30 meconium and 29 neonatal urine specimens were analyzed by GC/MS and found not to differ in sensitivity.[96] Moriya et al. and Wingert et al. reported the same outcome in their analysis of 50 and 345, respectively, meconium and neonatal urine specimens by EMIT and GC/MS.[109,110] These studies have one important analytical consideration in common; the use of equivalent analytical methods and cutoff concentrations to analyze both meconium and urine. In contrast, most studies reporting meconium to be more sensitive than urine have used a more sensitive analytical technique on the meconium specimens.[74] The one exception to this is a study conducted by Ryan et al., in which 100 meconium/neonatal urine pairs were analyzed by FPIA and GC/MS and the meconium was found to be more sensitive than the neonatal

urine in detecting prenatal cocaine exposure.[56] On the other hand, this same study also analyzed 737 maternal urine/meconium pairs and found no significant difference in detection rates among specimen types.

Ostrea et al. made the observation that the concentrations of analytes were higher in meconium based on the analysis of 5 meconium/neonatal urine pairs by RIA.[74] Similarly, Casanova et al. reported that meconium specimens collected within 24 hours of delivery were more likely to contain higher concentrations of cocaine and ecgonine methyl ester than neonatal urine.[96] In addition, they observed an increase in benzoylecgonine concentrations in meconium specimens collected 24-36 hours post-partum. They concluded that the increase in benzoylecgonine concentrations was probably due to urinary contamination of the meconium.[96]

In contrast to the studies mentioned previously, the current study found urinary concentrations of cocaine analytes to be significantly greater than concentrations found in meconium from matched pairs (Figure 4-4). Comparable to the work of Ostrea et al. and Casanova et al., the current study did not ensure that meconium specimens were not contaminated with urine.[74,96] Therefore, the true concentrations of cocaine analytes found in meconium may actually have been lower than detected. In the only other comparison study to report analyte concentrations, Moriya et al. found neonatal urine to contain higher concentrations of benzoylecgonine, the only cocaine analyte measured

by them.[109] This is consistent with meconium research, which reports a greater amount of the parent analyte, cocaine, and less metabolites. In this current study, cocaine was found to be in significantly greater concentrations in meconium and benzoylecgonine was found to be in significantly greater concentrations in neonatal urine. There was not a significant difference in concentrations of the other cocaine analytes between the two specimen types.

Recently, a minor adult urinary metabolite, *m*-hydroxybenzoylecgonine, has been reported to be a major contributor to the immunoreactivity in assays for benzoylecgonine in meconium.[13,32] Further analysis showed that 95% of screen positive meconium specimens contained *m*-hydroxybenzoylecgonine and that in 23% of the specimens this was the only analyte present. The authors concluded that assays that do not include this analyte run the risk of a high false negative rate.[13] In this current study one meconium specimen (5%) contained only *m*-hydroxybenzoylecgonine and no other analytes. Additionally, in 7 (35%) specimens *m*-hydroxybenzoylecgonine was not detected at all.

Unlike the studies by Moore et al. and Steele et al., which did not look at neonatal urinary concentrations of *m*-hydroxybenzoylecgonine, the current study found 74% percent of urine specimens to contain this analyte.[13,32] The concentrations of this analyte in urine were lower than ecgonine methyl ester but greater than cocaine. Based on these data, it would seem that

m-hydroxybenzoylecgonine, may be a metabolite produced in greater concentrations by the fetal/neonatal metabolic system and is not a metabolite unique to meconium specimens.

Many researchers have suggested that meconium serves as a reservoir into which drugs are deposited throughout gestation, and therefore, this specimen extends the window of detection of drug use to the last twenty weeks of gestation. The data generated by the current study do not necessarily support this theory, as only three specimens had discrepant results and the differences in the data were not statistically significant. The one urine specimen that was positive while the corresponding meconium was negative, had a maternal drug history claiming heavy drug use throughout gestation and last drug use three days before parturition. The two positive meconium specimens that had corresponding negative urine specimens had maternal histories claiming last use of 49 and 102 days before parturition (Table 4-6). In all other cases the results were concordant regardless of when the last reported use occurred.

Finally, when discussing the disadvantages of using neonatal urine to detect prenatal cocaine exposure, many researchers claim that, like adult urine, neonatal urine will also be negative if the mother has abstained from drug use for a few days. The data generated in this study is in direct conflict with this

generalization, with 53% of positive neonatal urine specimens coming from neonates whose subject drug histories were greater than 3 days since last use.

In conclusion, meconium specimens are easy to collect but difficult to manipulate and test. Neonatal urine collection is difficult and unreliable as collection bags can become dislodged and can irritate infant skin, but urine is easy to manipulate and test. Neither specimen presents a statistical advantage in detecting prenatal cocaine exposure, and the variable detection of drugs in these specimens may be due to individual differences in metabolism and clearance rather than differences in the detection abilities of the specimens.

CHAPTER 5
IS THERE A "GOLD STANDARD" FOR DETERMINING PRENATAL COCAINE EXPOSURE?

Introduction

Currently, analytical methods for identifying cocaine exposed neonates are inadequate and select for heavy and recent drug users. This lack of a "gold standard" causes misclassification of cocaine exposed neonates and threatens the validity of developmental findings, either by selecting for heavy users or by including users with nonusers. Additionally, unidentified cocaine exposed neonates would not receive interventional support that might be needed to compensate for detrimental effects of cocaine exposure.

The most commonly used specimens to ascertain cocaine use is neonatal or maternal urine, while meconium is the most frequently used alternative specimen. Specimens reported less often include amniotic fluid, umbilical cord tissue, colostrum, and maternal hair. Carefully conducted maternal histories are also used to identify potential drug users.

All of the aforementioned specimens and techniques have advantages and disadvantages. Although urine specimens are easy to analyze, they can be

difficult to collect. On the other hand, meconium, hair, and cord specimens are easy to collect, but require extensive manipulation for analysis. Colostrum specimens, much like urine specimens, are difficult to collect but analysis is relatively simple. It is also reported that colostrum collection is uncomfortable for the donor and only limited amounts are collected. Like colostrum specimens, analysis of amniotic fluid specimens is relatively simple. However, the timing for the collection of amniotic fluid specimens can be tricky, especially if collection is from the vaginal pool. Maternal histories can provide useful information about amounts and timing of drug use, but must be conducted in a carefully controlled, non-threatening manner. In particular, interviews are often plagued by denial, which is characteristic of drug dependent individuals.

The one disadvantage shared by all of these methodologies is high false negative rates. Urine and meconium have reported false negative rates of 22-60% and 20-54%, respectively.[74] Maternal histories alone, can misclassify 10-60% of drug users.[115] Studies of maternal hair have the lowest reported false negative rates, ranging from 3-25%.[73] While it is clear that no single method or specimen is matchless for detecting prenatal cocaine exposure, combinations of methods and specimens may form a definitive measure for detecting prenatal cocaine exposure, by compensating for inadequacies inherent in each specimen or method.

Previous studies comparing the sensitivities of various specimens for identifying prenatal cocaine exposure have been plagued by several difficulties,

including; small sample size, the use of different cutoff values and methodologies when analyzing specimens, failure to use paired or matched specimens, and the absence of statistical analyses (Chapter 4). Moreover, none of these studies attempted to use combinations of specimens to improve overall sensitivity for detecting prenatal cocaine exposure. This chapter will use the data generated in this study to: compare the sensitivities of the various specimens, define combinations of analytes and specimens which may be more likely to detect prenatal cocaine exposure, and discuss the possibility of defining a benchmark or "gold standard" for detecting prenatal cocaine exposure.

Materials and Methods

The subjects, specimens, materials, and methods used to detect cocaine and its metabolites have been described in the preceding chapters.

Results and Discussion

Maternal History and Subject Results

An important consideration when evaluating specimens for detecting prenatal cocaine exposure is the ability of a specimen to reflect drug use that is sporadic or not recent. Subjects who use drugs in small amounts, use drugs sporadically, or abstain from drug use shortly before delivery, are less likely to

be detected than frequent and recent users. The distribution of specimens with respect to the reported number of days since last cocaine use and the percent positive in each category is presented in Table 5-1. In general, greater than 80% of all specimens were positive for cocaine analytes if use occurred within 7 days of parturition. As expected from specimens which are subject to biological clearance of drugs over time, there is a general decrease in the percent of specimens testing positive as the number of days since last drug use increases for meconium, urine, and umbilical cord tissue. In contrast, the percentage of positive hair specimens was relatively constant over time. Colostrum and amniotic fluid positives had no discernible pattern in the percentage of positives over time. This may be due to the small number of specimens, resulting in an inability to minimize effects from the tendency of some subjects to report drug use that occurred in the far past and deny recent drug use. [10,115]

Analyte Distributions in Positive Specimens

Another important consideration is the distribution of analytes that are found in the different specimen types. For example, screening methods that are selective for cocaine would be less effective on specimens that contain greater concentrations of other cocaine analytes. The distribution of cocaine analytes found in each specimen type is presented in Table 5-2. Benzoylecgonine was the most common analyte detected in all specimens except colostrum, and was present in 100% of all specimen types except meconium and colostrum.

Cocaine was present in 100% and 75% of colostrum and meconium specimens, respectively.

According to the results of this study, screening methods that are selective for benzoylecgonine would be the most effective for all specimen types except colostrum. This is not necessarily in agreement with other studies, which have shown cocaine to be the most common analyte detected in meconium and hair.[73] This study did not ensure that meconium specimens were not contaminated with neonatal urine, but in studies where urine free meconium was analyzed, cocaine was found to be the most common analyte.[13,74] The results of the urine free meconium studies, while useful in establishing information about fetal metabolism, is not necessarily applicable for deciding which analyte to measure in a hospital setting, as any meconium specimens taken from the nursery for the purpose of screening for drugs of abuse are likely to be urine contaminated. The implications of hair specimens that contain only benzoylecgonine are discussed in Chapter 2.

Overall Sensitivity of Specimens

The most important consideration when evaluating specimens for detecting prenatal cocaine exposure is the sensitivity of the specimen for detecting drug use. Sensitivity (detection efficiency) was defined as:

$$S = \frac{\text{Total number of positive specimens}}{\text{Total number of target specimens + Number of control specimens testing positive}}$$

The overall sensitivities of the various specimens is presented in Figure 5-1. Hair had the best overall sensitivity, followed by maternal history, colostrum, amniotic fluid, meconium, urine, and umbilical cord tissue. There have been no thorough investigations of the sensitivity of colostrum and umbilical cord tissue reported in the literature, therefore, it is difficult to speculate as to the usefulness of these specimens in determining prenatal cocaine exposure. Moreover, the small number of colostrum specimens in contrast to the numbers of other specimens in this study (see: Chapter 1, Table 1-2) further complicates the usefulness of comparing the sensitivity of colostrum with the other specimens in the study. Amniotic fluid specimens have reported sensitivities of 50-74%, which compares with the 60% sensitivity determined in this study.[13,96] Meconium sensitivities have been reported in the range of 52-80%.[73,74,96] The 55.6% sensitivity of meconium in this study is consistent with the lower end of this range. Likewise, urine sensitivities have been reported to be 30-69%, which compares to the 54.3% sensitivity of this study.[16,73,74,96] Reported hair sensitivities range from 70-100%, which is consistent with the 77.4% sensitivity in this study (Chapter 2). Maternal drug histories have a wide range of reported sensitivities from a low of 25% to a high of 100%.[115] The sensitivity of maternal history is heavily dependent on the study population, with

lowest sensitivities exhibited by low-risk suburban populations and the highest sensitivities found in drug treatment facilities.[115] The maternal history sensitivity of 75.5% in this study is consistent with a study population in which target subjects were already identified by the hospital staff as drug users, and therefore would not suffer any additional negative consequences for admitting drug use in this study.

Intraindividual Comparison of Specimen Results and Statistical Analysis

The above sensitivity data included all available specimens in the comparison of sensitivities of the different specimen types. It is difficult, though, to draw conclusions as to whether the differences noted between the specimen types are significant because the total number of specimens in each category is not the same (i.e. specimens are not matched). A more rigorous evaluation of the sensitivities of the different specimens would be to compare the results of different specimens taken from the same individual, or as in this study mother-infant pair. McNemars Test, Exact Method was performed on all matching pairs of specimens to determine if there are intraindividual differences in the ability of the various specimens to detect prenatal cocaine exposure.[89] The results are presented in Table 5-III. The smallest p-values were noted for the comparison of any specimen to its corresponding maternal hair specimen, but only the comparison of umbilical cord tissue to hair showed hair to be significantly more likely to detect prenatal cocaine exposure ($p=0.04$).

Only a few studies have used rigorous statistical methods to analyze intraindividual differences in the ability of different specimens to reflect cocaine use. Callahan et al., compared matched meconium, infant urine, and hair specimens and found maternal hair to be the most sensitive for detecting prenatal cocaine exposure.[73] The findings in the Callahan et al. study though, must be viewed with caution as each specimen type was analyzed with a different analytical method.[73] Therefore differences in sensitivity may reflect differences in the methods rather than the specimens themselves. Casanova et al. compared the GC/MS results from 30 matched meconium and neonatal urine specimens and found no difference between the sensitivities of the two specimens.[96] In comparison, Ryan et al. analyzed 100 matched meconium and neonatal urine specimens by FPIA and GC/MS and found meconium to be more sensitive than neonatal urine for detecting prenatal cocaine exposure.[56]

There may be several reasons why few intraindividual differences between specimens were found in this study. First, there may be no differences. Second, differences in specimen sensitivity may be too small to detect with the number of matched pairs available. Finally, the target population was not evenly distributed with respect to the amount of time elapsed since the last cocaine use. Greater than 75% of the subjects continued to use cocaine in the last half of pregnancy and only 17.7% and 35.4% of subjects stopped use of cocaine after the first and second trimesters, respectively (Table 5-2). Hence, the target subject population was enriched in recent drug users and differences in the

ability of specimens to detect cocaine use that is not recent, is overwhelmed by the high number of concordant specimen results that is observed when drug use is current..

Is there a "gold standard"?

It is clear from the results of this study that no single specimen or method is able to identify all instances of drug use and thus none qualify as a "gold standard." Collated results of all positive specimens is presented in Table 5-4 and a summary of all specimen results in presented in Table 5-5. Maternal history provided a high degree of sensitivity, with only 11 of 49 (22%) controls (subjects denying use), subsequently testing positive for cocaine analytes in one or more specimens. Moreover, 7 of 34 (20%) targets (subjects admitting cocaine use) were not detected by any specimen tested and would have been missed without an interview. These results underscore the need for carefully designed interviews conducted by a skilled interviewer or nursing professional when attempting to identify drug users.

The four specimen types with the highest total number of specimens tested are urine (75), meconium (74), umbilical cord tissue (70), and maternal hair (66). Among these four specimen types, maternal hair was the specimen with the best record at identifying positives, detecting cocaine analytes in 19 of 26 (73%) targets tested and 5 of 40 (12%) controls tested. Meconium was the

next best with 16 of 32 (50%) targets and 4 of 42 (9%) controls identified as positive. Neonatal urine had a record very similar to meconium, identifying 15 of 31(48%) targets and 4 of 44 (9%) controls as positive. Finally, umbilical cord tissue had the worst record identifying only 12 of 28 (43%) targets and 1 of 42 (2%) controls.

Eleven specimens from control subjects were identified as positive by one or more of the specimen types (Table 5-4). Four of the 11 (36%) were positive by two or more specimen types. Maternal hair identified the most specimens with 5 of the 11 (45%) control specimens. In addition, hair had high concordance with other specimen types, with 3 of the 5 (60%) identified as positive by at least one other specimen type. Meconium and urine each identified 4 of the 11 (36%) control specimens. Concordance among the meconium specimens was the lowest with only 1 of 4 (25%) identified as positive by at least one other specimen type. Conversely, urine had the highest concordance with other specimen types with 3 of 4 (75%) identified as positive by at least one other specimen type.

Given the above discussion, the best combination for identifying prenatal cocaine exposure would be maternal interview and the specimen type that identifies the most positives. Using all specimen results, the best combination is maternal history and maternal hair with a sensitivity of 86%. Combinations of maternal interview with meconium and maternal interview with urine both yielded a sensitivity of 85%. Combinations of maternal interview with the other

specimen types yielded sensitivities of 79% and 76% for amniotic fluid and umbilical cord tissue, respectively. There does not appear to be much difference between these various combinations. The small differences in sensitivity is probably due to the high sensitivity of the maternal interview, which may or may not be applicable to the population at large for reasons outlined earlier in this chapter.

Conclusion

In conclusion, maternal hair had the best overall sensitivity for determining prenatal cocaine exposure, but statistical analysis revealed that conclusions based upon general differences in specimen sensitivity can be misleading. Comparisons of different specimen types taken from the same maternal/neonatal pair revealed no intraindividual differences in the sensitivities of the various specimen types, with the exception of maternal hair and umbilical cord tissue. Combining maternal interview with specimen results improved sensitivities by 10-25%, but fell short of an ideal "gold standard". The only combination to yield a sensitivity of 100% was maternal interview, amniotic fluid, urine, meconium, and hair which, in a practical sense, is clearly not attainable. Future studies, with higher percentages of matched specimens may reveal significant differences between the specimen types in their ability to identify prenatal cocaine exposure.

Table 5-1. Percentage of positive specimens categorized by the number of days since last reported drug use,

Days Since Last Use	Meconium (n)	Urine (n)	Amniotic fluid (n)	Cord (n)	Hair (n)	Colostrum (n)
< 7	91.6 (12)	100 (11)	83.3 (6)	87.5 (8)	81.8 (11)	100 (2)
8-29	40.0 (5)	40.0 (5)	0 (1)	50.0 (4)	75.0 (4)	100 (2)
30-89	25.0 (4)	0 (4)	0 (3)	25.0 (4)	66.7 (3)	0 (1)
90-179	33.3 (6)	20.0 (5)	NA (0)	33.3 (6)	50.0 (4)	25 (4)
>180	0 (5)	16.7 (6)	66.7 (3)	0 (6)	75.0 (4)	100 (1)

Notes: The first number in each column is the percentage of positive specimens. The number in parentheses is the total number of specimens in that category.

Table 5-2. Analyte distribution in study specimens.

Specimen	n	EME %	EEE %	NCOC %	COC %	CE %	BE %	MOHBE %
Urine	19	68	0	11	47	0	100	74
Meconium	20	75	10	40	75	15	85	65
Cord	13	38	0	23	8	0	100	23
Hair	24	23	0	5	69	7	100	0
Amniotic fluid	9	56	11	11	11	0	100	33
Colostrum	6	67	0	17	100	0	67	0

Note: n= the number of positive specimens for any analyte.
Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

Table 5-3. Intraindividual comparison of specimens for detecting prenatal cocaine exposure and results of statistical analyses.

Specimen A	Specimen B	Specimen A		Specimen B		p
		+	-	+	-	
Urine	Meconium	16	51	18	49	0.76
Urine	Colostrum	3	16	5	14	2
Urine	Hair	17	42	22	37	0.23
Urine	Cord	15	49	12	52	0.51
Urine	Amniotic Fluid	7	25	9	23	0.69
Meconium	Colostrum	5	13	5	13	1.4
Meconium	Hair	17	41	22	36	0.33
Meconium	Cord	15	51	12	54	0.51
Meconium	Amniotic Fluid	10	24	9	25	1
Amniotic Fluid	Colostrum	2	4	2	4	2
Amniotic Fluid	Hair	6	19	9	16	0.45
Amniotic Fluid	Cord	9	25	9	25	0.69
Cord	Colostrum	5	12	5	12	1.3
Cord	Hair	10	44	18	36	0.04
Colostrum	Hair	5	12	5	12	1.5

Notes: Number of positive specimens (+) and number of negative specimens (-). Statistical analysis performed using the McNemar test, Exact Method and results considered significant if $p < 0.05$.

Table 5-4. Qualitative results of all specimens categorized by specimen type.

Specimen (ID)	History	Urine	Meconium	Hair	Cord	Amniotic Fluid	Colostrum
2	P	N	P		P		N
4	P	N	N	P	N	P	
5	P	N	N	P	N		
6	N		N		N	P	
8	P	P	P	P	P	N	
10	P	P	P	P	P	P	P
11	P	N	N		N		N
13	P	P	P		P	P	
14	P	N	N		N	P	P
16	N	N	N	P		N	
17	P	P	P	P			P
18	N	P	N	N*	P		
19	N	P	N	N	N		
20	P	N	N		P		N
23	P	P	P	P	P		
25	P	P	P	N	P	P	
27	P	N	N		N		
30	P	N	N	P	P		N
32	P		N	P	N	N	
33	P		N	N	N		
34	P	P	P	P	N		P
35	N	N	P	N	N	N	
41	P	P	P	P			
42	P		P	P			
45	P	N	N	N	N		
47	P	N	N	N	N	N	
48	P	N	N	N	N		P
52	P	P		P	N		
53	P	P	P	P	P	N	
54	P	N	P	N	N		
56	P	P	P	P	P	P	
57	P	N			N	N	
58	N	N	P	N	N		N
60	P	P	N	P	N		
61	P	P	P	N	P	P	
62	N	N	P	N	N	N	
65	P	P	P	P	P		P
68	P	N	N	P			
71	N	P	N	P	N		
72	N	N	N	P	N	N	
73	N	N	P	P	N	P	
75	N	P		P	N		
76	P	P	P	P			
78	P	N	N	N	N	N	
85	P	N	N	P			

Note: P=postitive and N=negative

* hair specimen was negative but wash specimen was positive.

Table 5-5. Distribution of positive targets and controls by specimen type and maternal history.

	History	Urine	Meconium	Hair	Cord	Amniotic Fluid	Colostrum
Targets (n)	34	31	32	26	28	13	10
% Positive		48	50	73	43	54	60
Controls (n)	49	44	42	40	42	19	11
% Positive		9	9	12	2	10	0

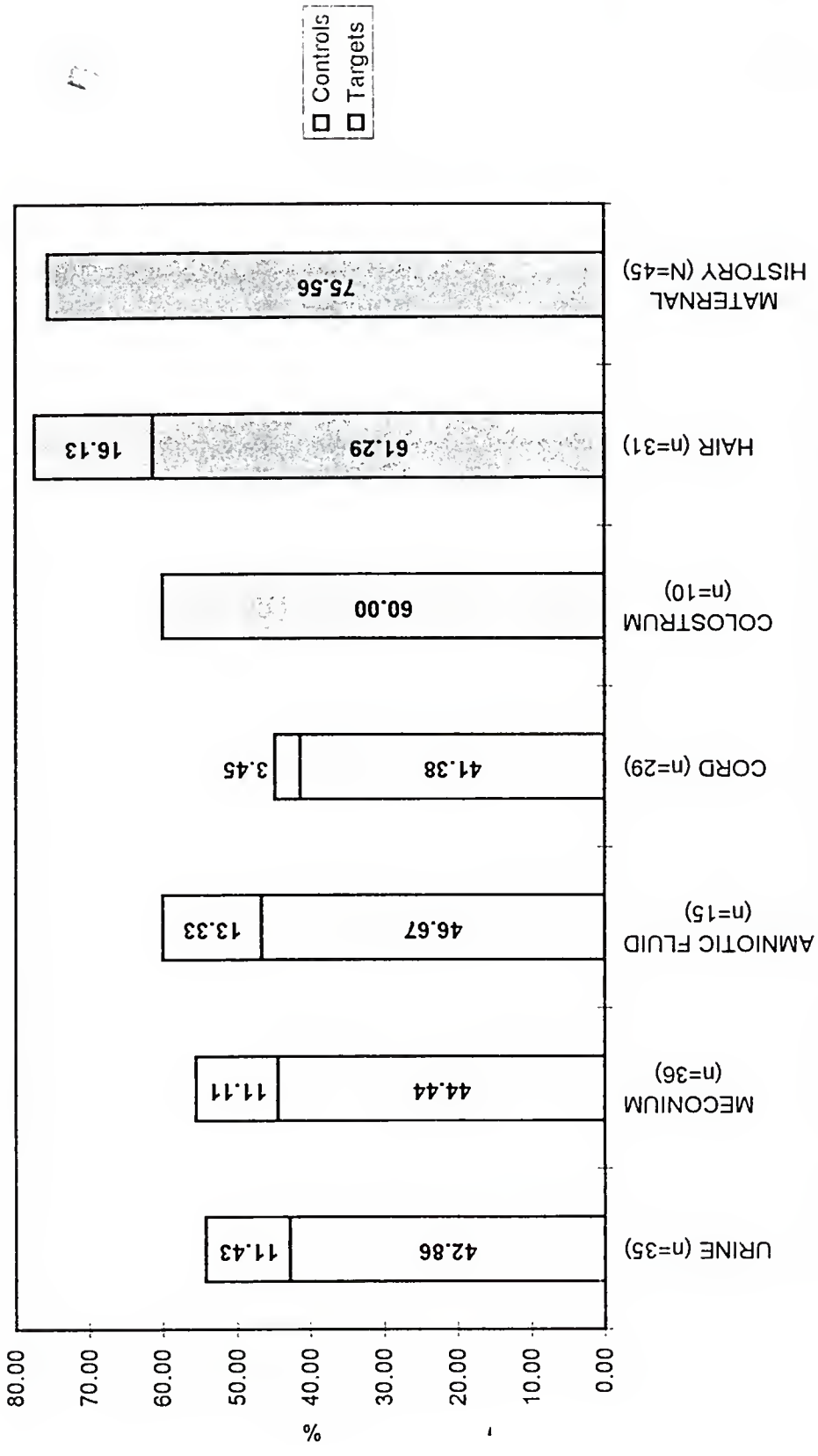


Figure 5-1. Sensitivity of Specimens for Detecting Prenatal Cocaine Exposure. n= total number of target specimens + the number of control specimens testing positive.

CHAPTER 6
CONCLUSIONS AND FUTURE WORK

This dissertation has described analytical methods for the detection of cocaine, and the cocaine metabolites; benzoylecgonine, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, *m*-hydroxybenzoylecgonine, and norcocaine in maternal hair, neonatal urine, meconium, umbilical cord tissue, amniotic fluid, and colostrum.

It is believed that this report is the first to describe the detection of *m*-hydroxybenzoylecgonine in neonatal urine, and a comparison of the available data to the results of this metabolite in meconium (Chapter 4). Also in Chapter 4, is the first report of ecgonine ethyl ester in meconium. A number of other "firsts" were described in Chapter 3, including the detection of cocaine, benzoylecgonine, norcocaine, and ecgonine methyl ester in colostrum, the detection of *m*-hydroxybenzoylecgonine and ecgonine ethyl ester in amniotic fluid, and the detection of *m*-hydroxybenzoylecgonine in umbilical cord tissue. Future studies need to include these analytes to assess the implications of the

presence of these metabolites on what is known about maternal-fetal metabolism.

In Chapter 2, the description of the use of maternal hair for detecting gestational cocaine use is one of only two reports to segment maternal hair into sections representative of the different trimesters. Both studies showed that hair sections representative of the third and second trimesters had the highest sensitivity, while hair representative of the first trimester had lower sensitivity. This is consistent with the general view that hair washing and chemical treatments can leach drugs from hair over time. Despite the lower sensitivity of specimens representative of the first trimester, the results of this study indicate maternal hair has potential for detecting prenatal cocaine exposure. However, the small number of hair specimens collected from subjects who admitted to cocaine use, and the lack of a significant number of specimens from subjects who used cocaine only during the first half of pregnancy, make it difficult to draw any conclusions about the real potential of maternal hair for detecting prenatal cocaine exposure.

Future studies investigating the use of maternal hair for determining prenatal cocaine exposure should include more subjects that had used cocaine exclusively in the first and second trimesters. Information concerning the hair care habits of the study population, particularly the use of chemical hair treatments, should be collected and compared to the analytical results.

Two main observations were drawn from the results of the work presented in Chapter 3. First, amniotic fluid holds some potential as an alternative to neonatal urine for the detection of prenatal cocaine exposure. The small number of specimens though, makes it difficult to draw a definitive conclusion in this matter. Second, the analysis of colostrum showed the potential for delivering a dose of cocaine, through breast feeding, that could elicit physiological effects in an infant. Future studies should include greater numbers of subjects.

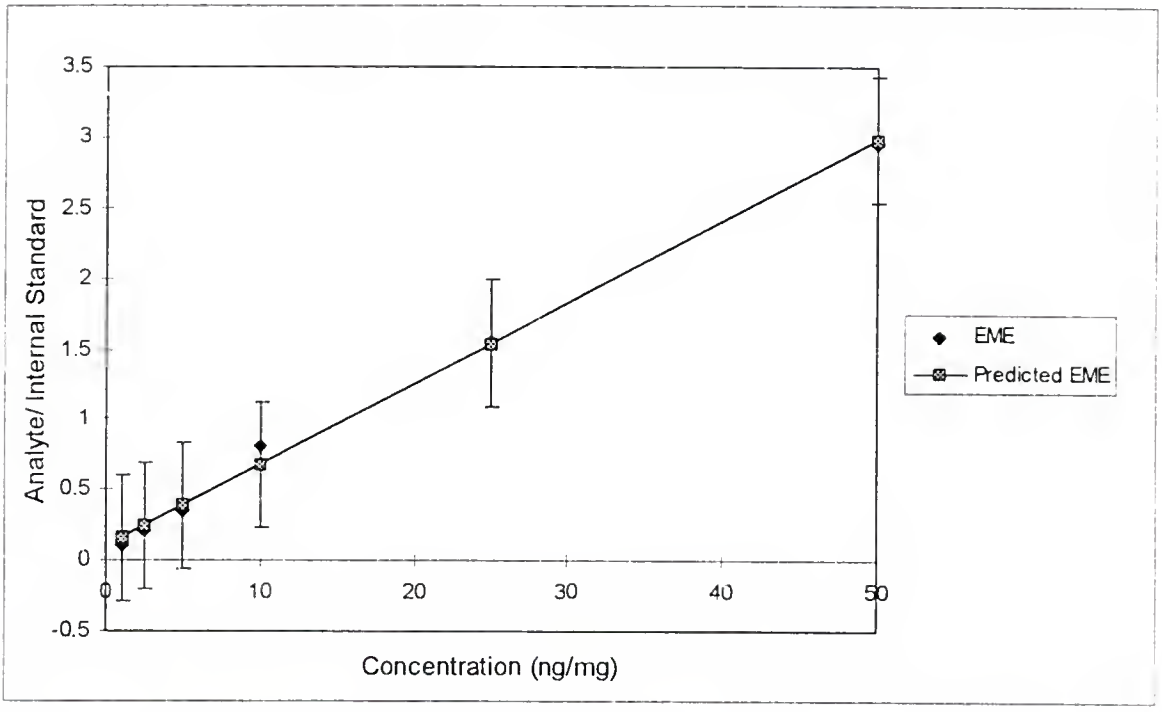
Many researchers have claimed meconium to be a superior specimen to urine for the detection of prenatal cocaine exposure. Chapter 4 examined this claim, and concluded that when rigorous statistical analyses are undertaken, apparent differences in sensitivity between the two specimens are not significant. In addition, use of an immunoassay screening procedure with SAMHSA recommended cutoff concentrations of 300 ng/mL is not effective for identifying cocaine metabolites in neonatal specimens. Moreover, lowering the cutoff to 75 ng/mL resulted in an increase in the identification of cocaine exposed subjects but the majority of these subjects remained undetected, even at this lower cutoff concentration. Future studies should be wary of making comparisons based on sensitivities or numbers alone, and should be sure to analyze all specimens with the same analytical method and cutoff values.

Chapter 5 compared the results from all of the specimens in this study and found that, with the exception of maternal hair over umbilical cord tissue, any specimen examined in this study is as likely as any other to detect prenatal cocaine exposure in the same individual. This conclusion, though, is not applicable to the population of pregnant women at large as the majority of the specimens taken from admitted drug users in this study were from subjects who had recently used drugs. In order to define the true ability of a specimen to reflect any drug use, a greater percentage of the study population needs to be comprised of subjects who only used drugs during the first half of pregnancy.

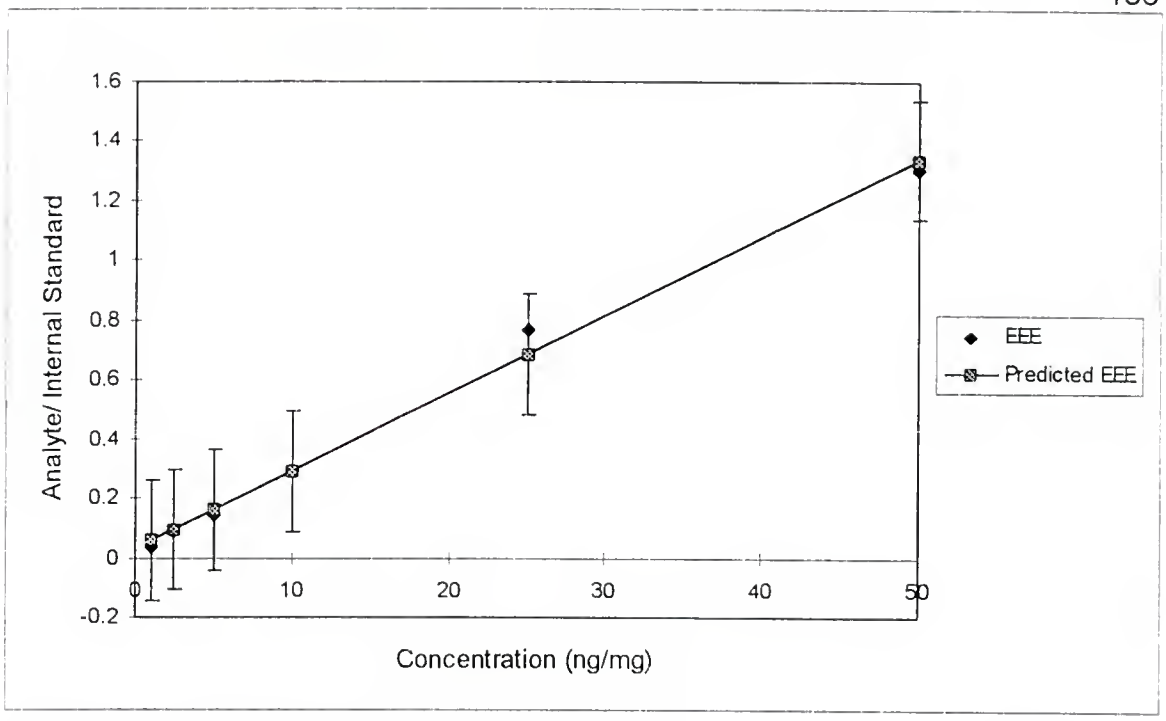
Finally, the results of this study make it clear that no single specimen has the ability to reflect prenatal cocaine use in100% of the cases. This is unfortunate as it means that misclassification of cocaine exposed neonates in health outcome research will continue to be a problem and that neonates in need of interventional support may not receive the care they need.

APPENDIX I: GC/MS CALIBRATION CURVES

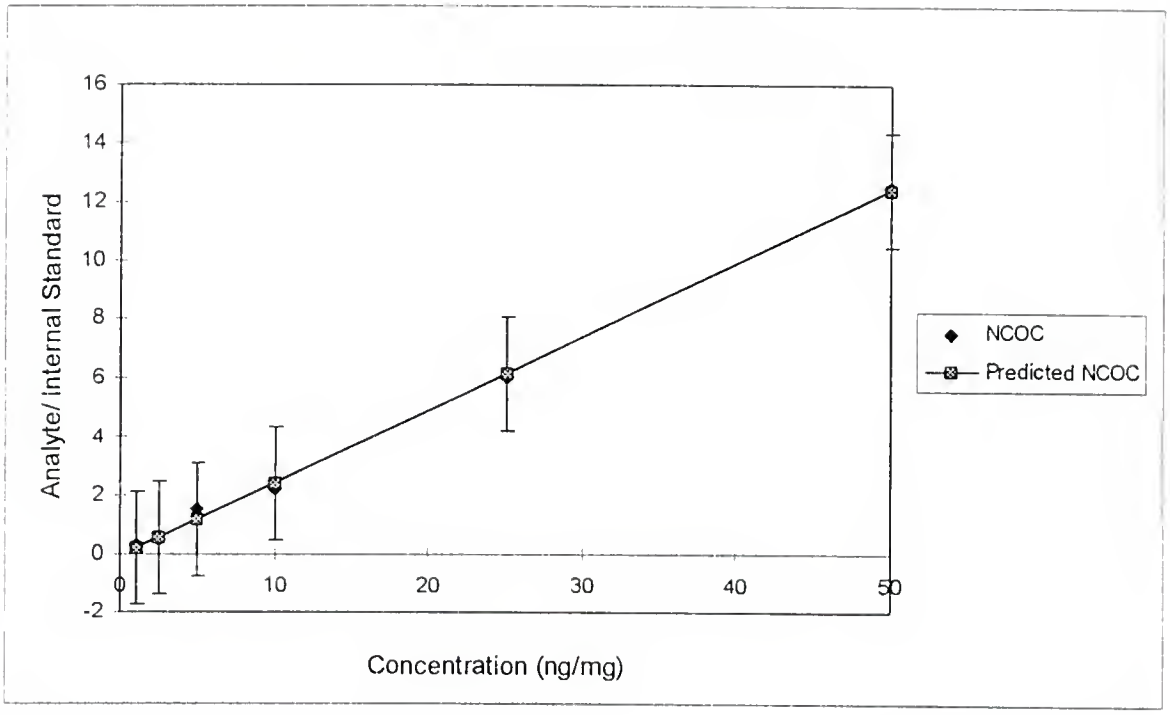
Representative GC/MS calibration curves for each analyte in each matrix. Curves are plotted as ratio of analyte quantitation ion to internal standard quantitation ion versus concentration. Curves include actual points, regression analysis predicted points and standard error bars. In linear curves, all points will fall within the standard error area.



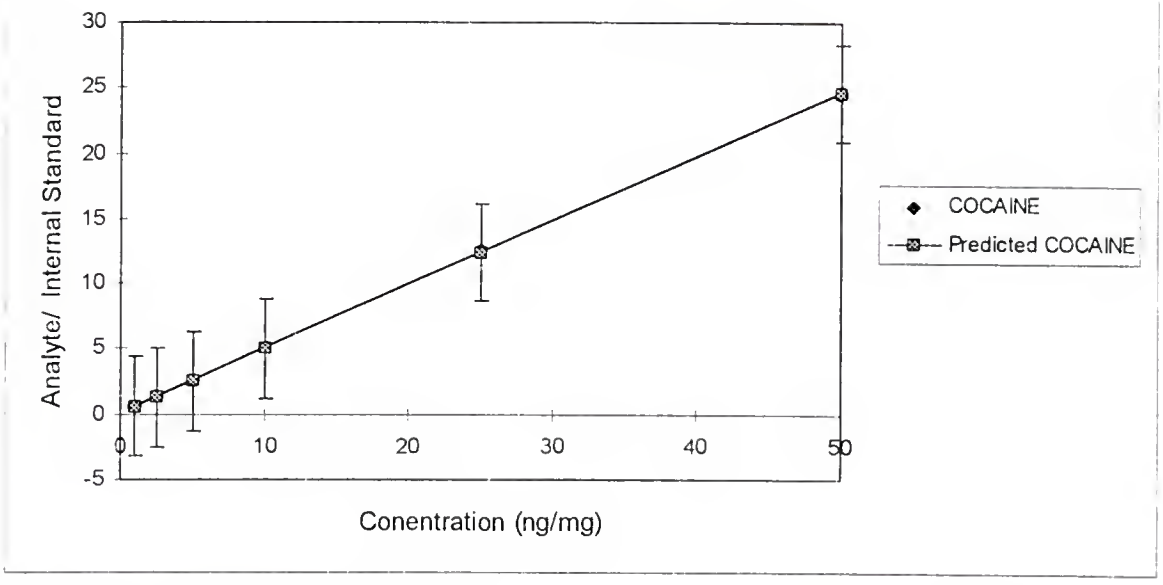
Curve I-1. Ecgonine Methyl Ester (EME) in Hair.



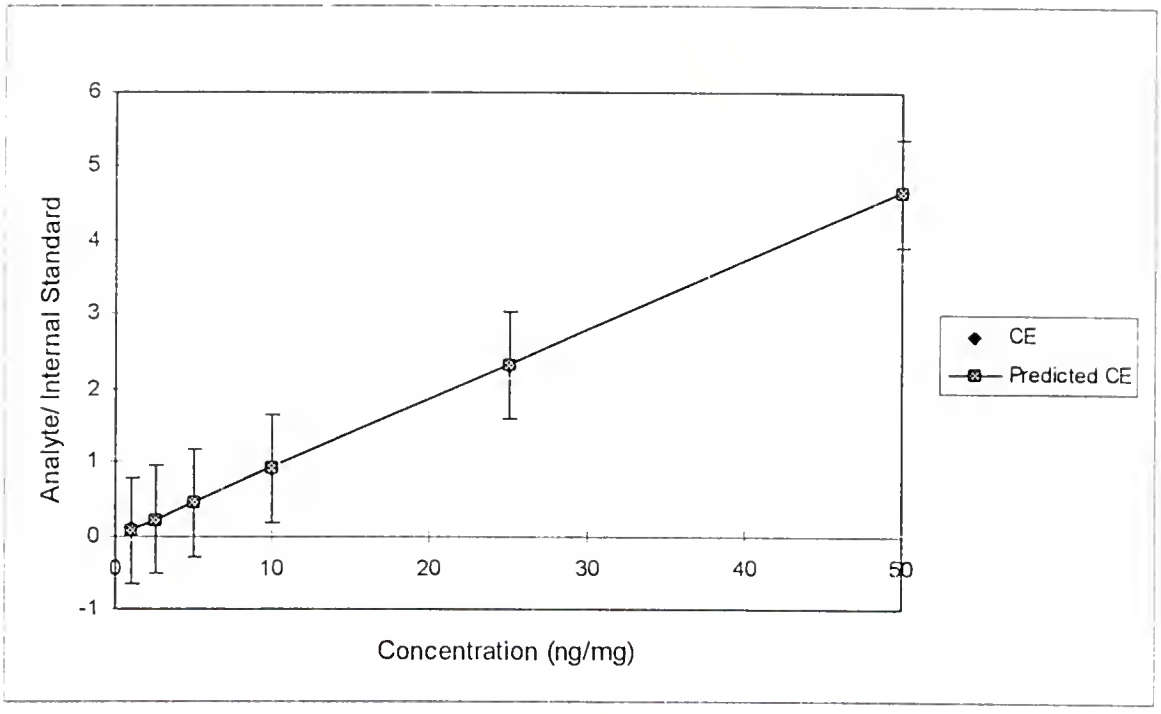
Curve I-2. Ecgonine Ethyl Ester (EEE) in Hair.



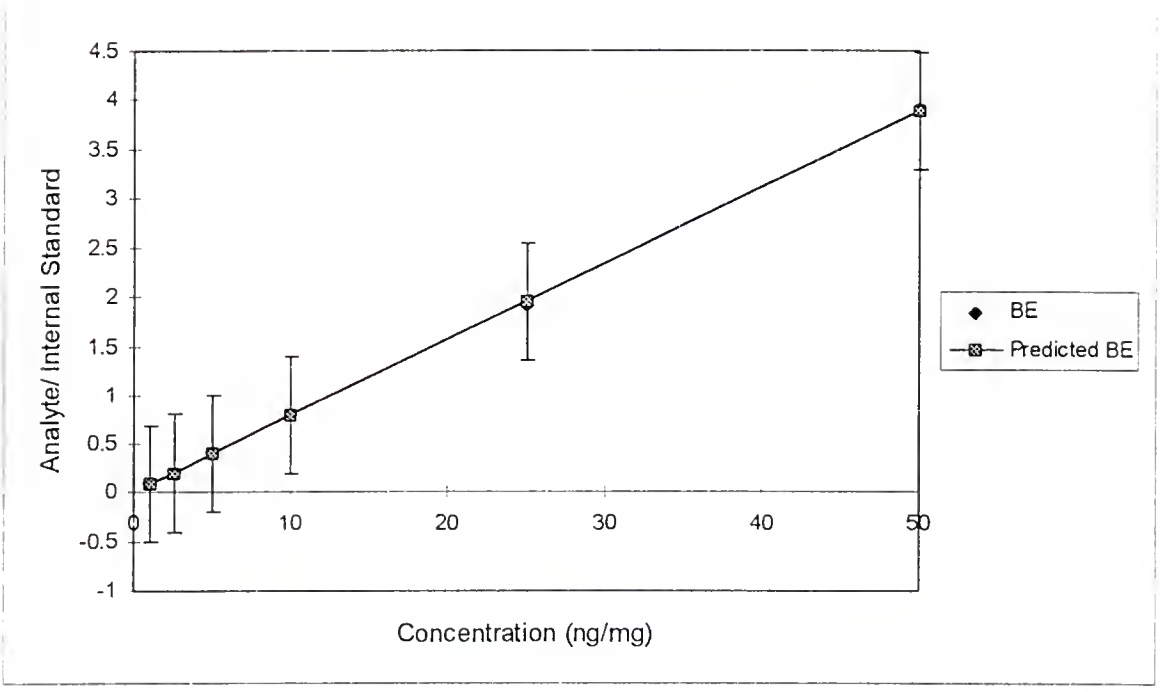
Curve I-3. Norcocaine (NCOC) in Hair.



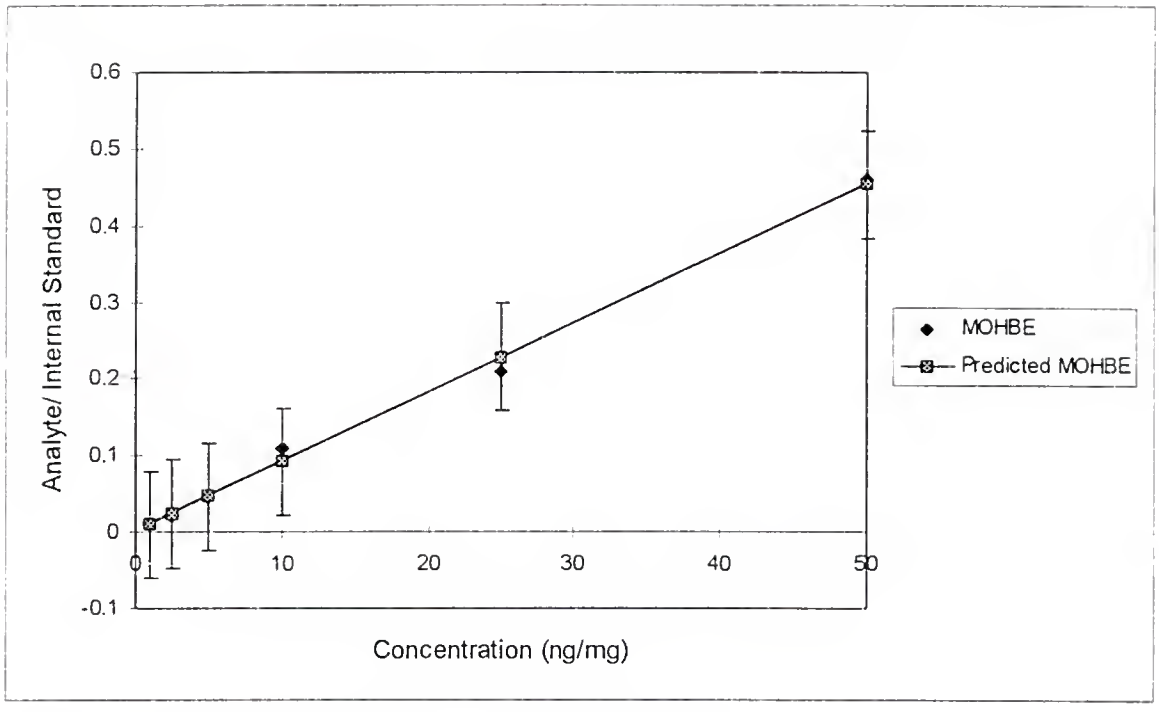
Curve I-4. Cocaine (COC) in Hair.



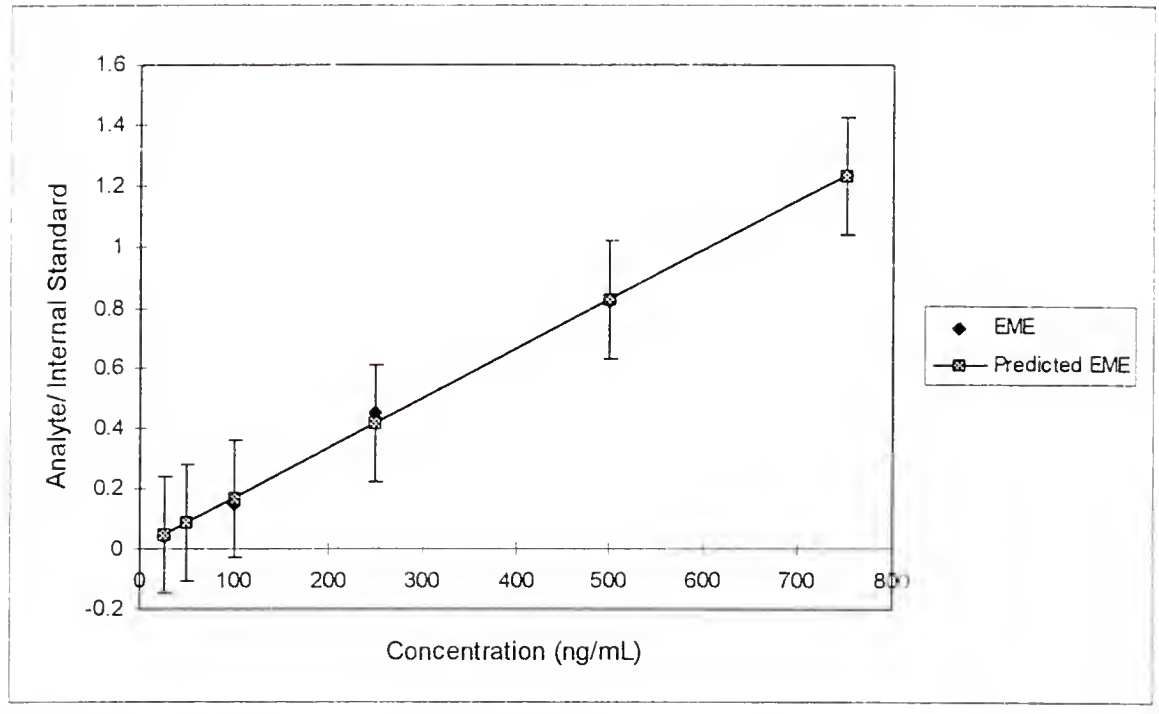
Curve I-5. Cocaethylene (CE) in Hair.



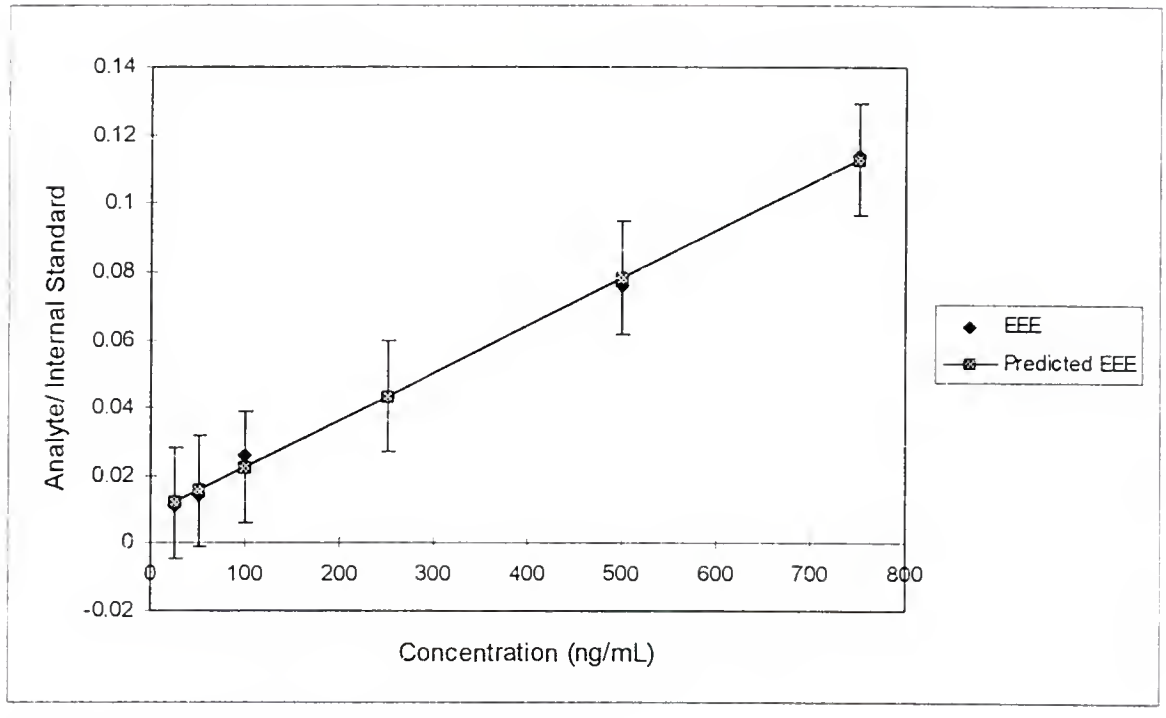
Curve I-6. Benzoylecgonine (BE) in Hair.



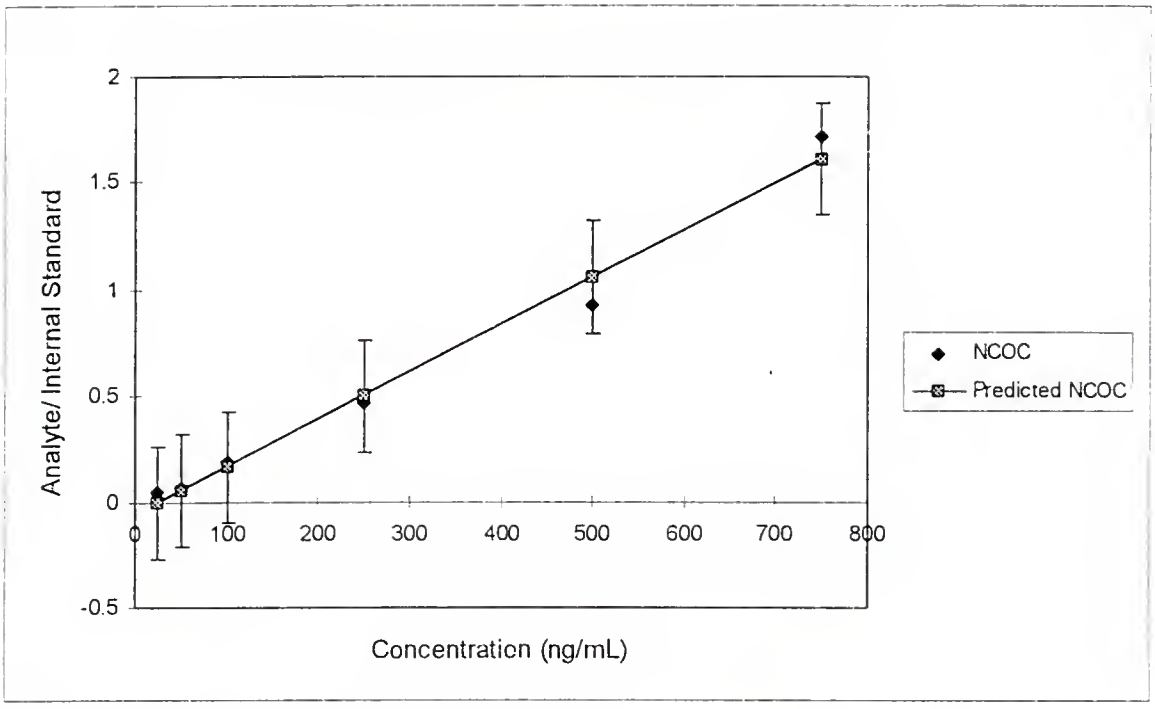
Curve I-7. *m*-Hydroxybenzoylecgonine (MOHBE) in Hair.



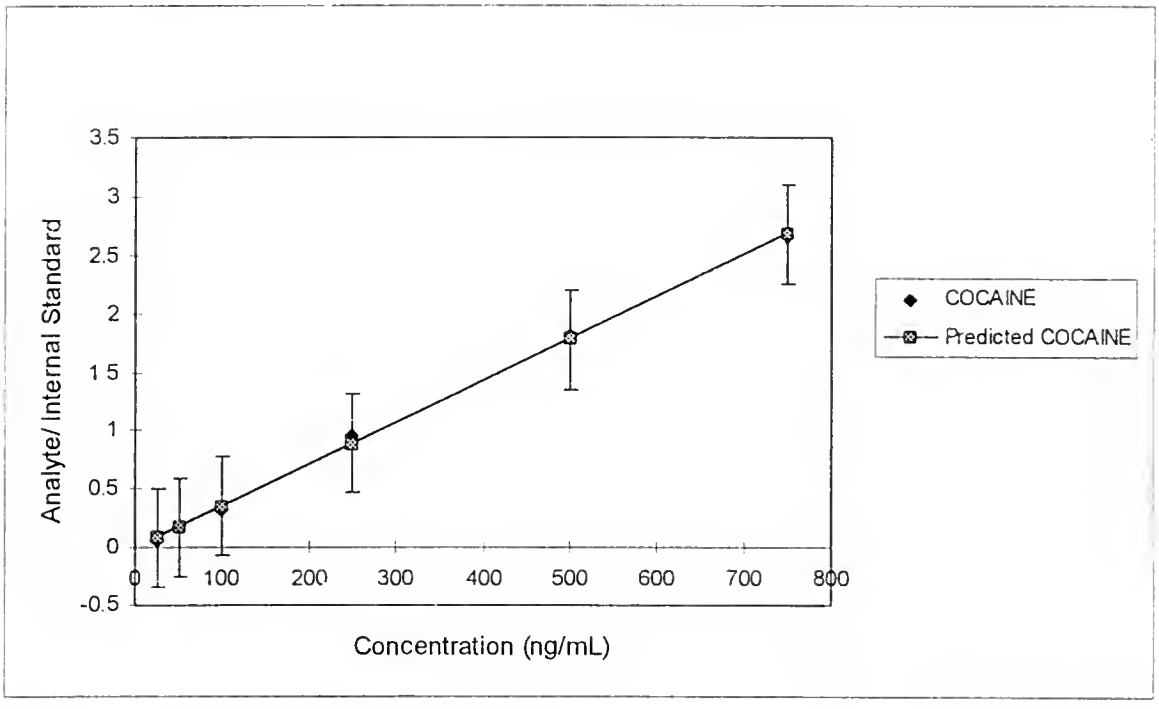
Curve I-8. Ecgonine Methyl Ester in Urine.



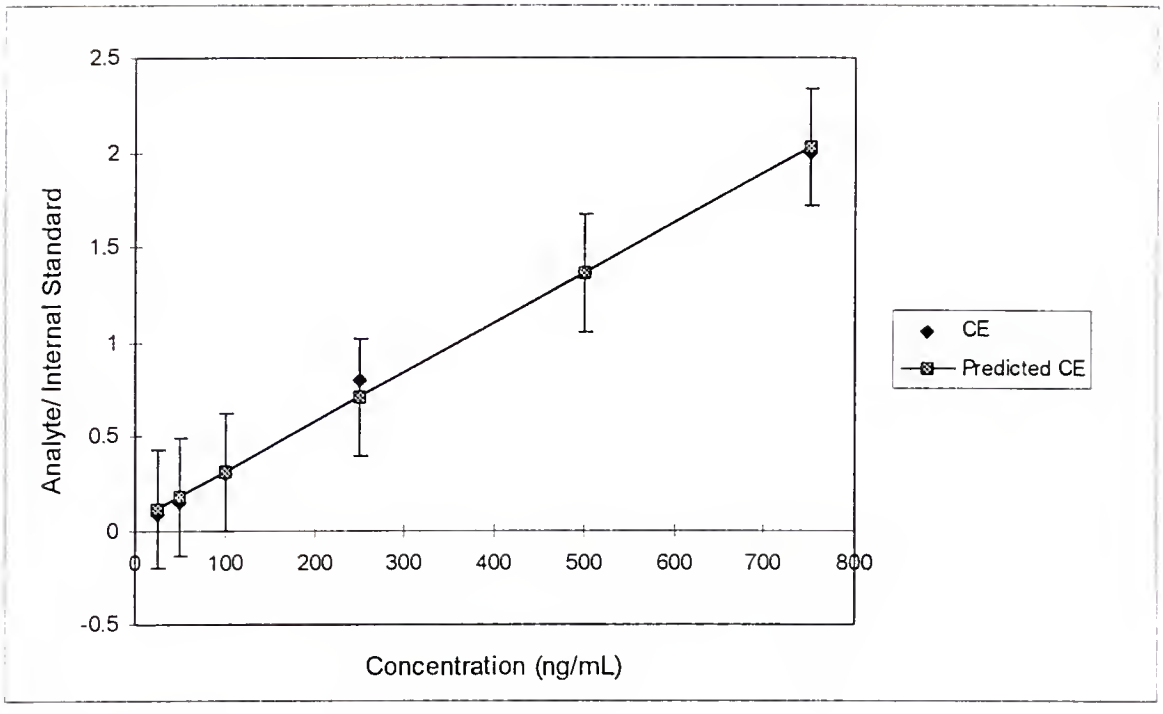
Curve I-9. Ecgonine Ethyl Ester in Urine.



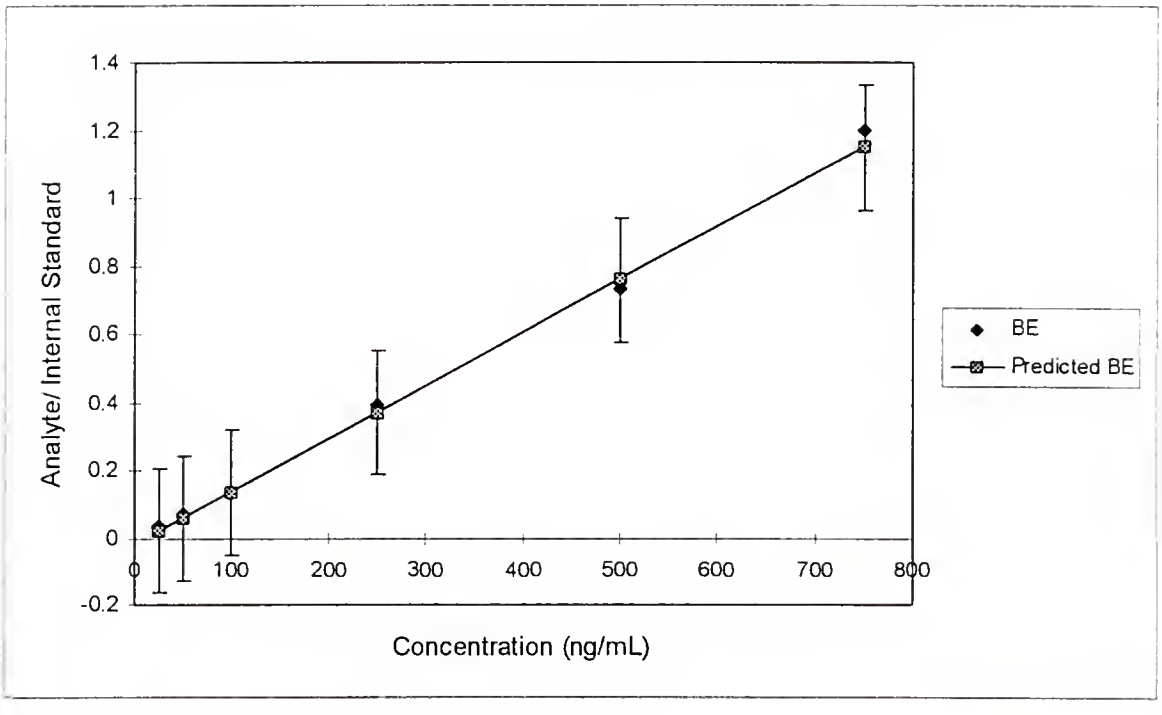
Curve I-10. Norcocaine (NCOC) in Urine.



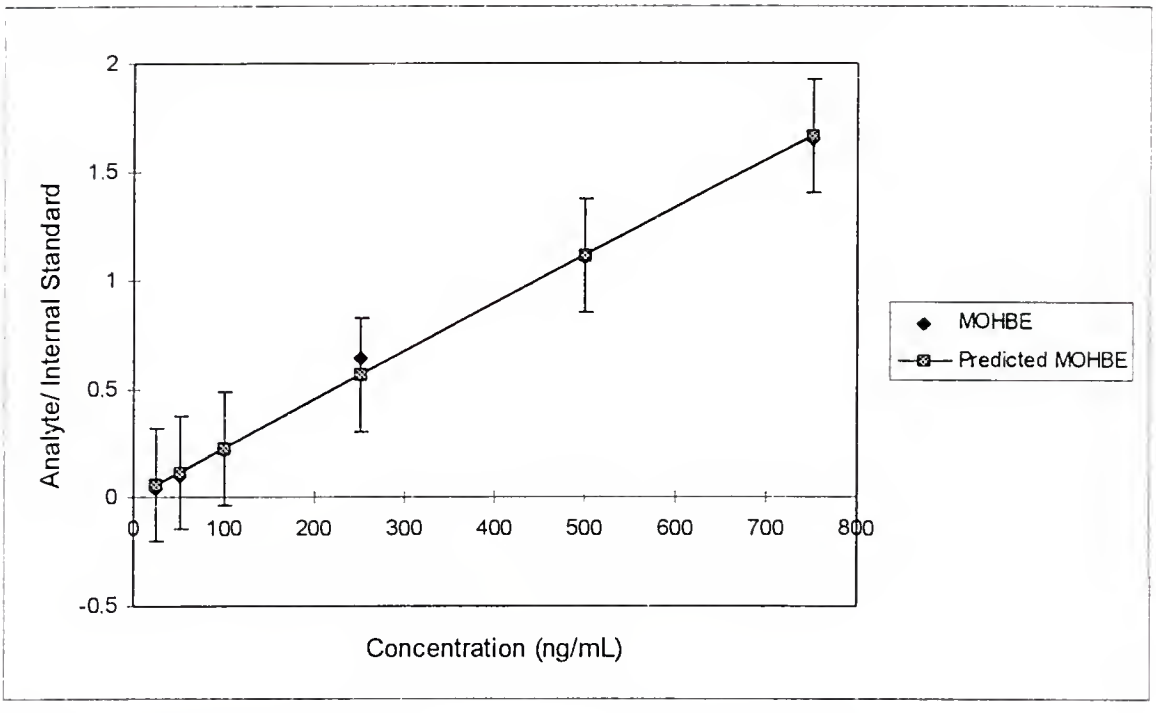
Curve I-11. Cocaine (COC) in Urine.



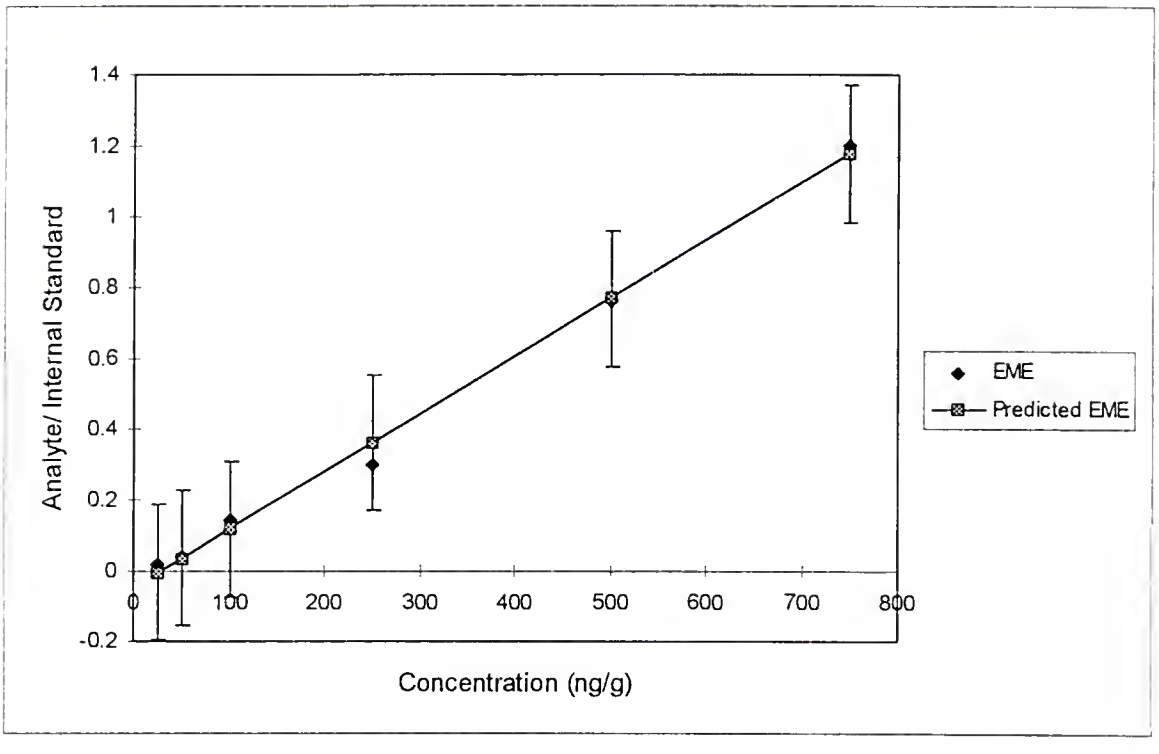
Curve I-12. Cocaethylene (CE) in Urine.



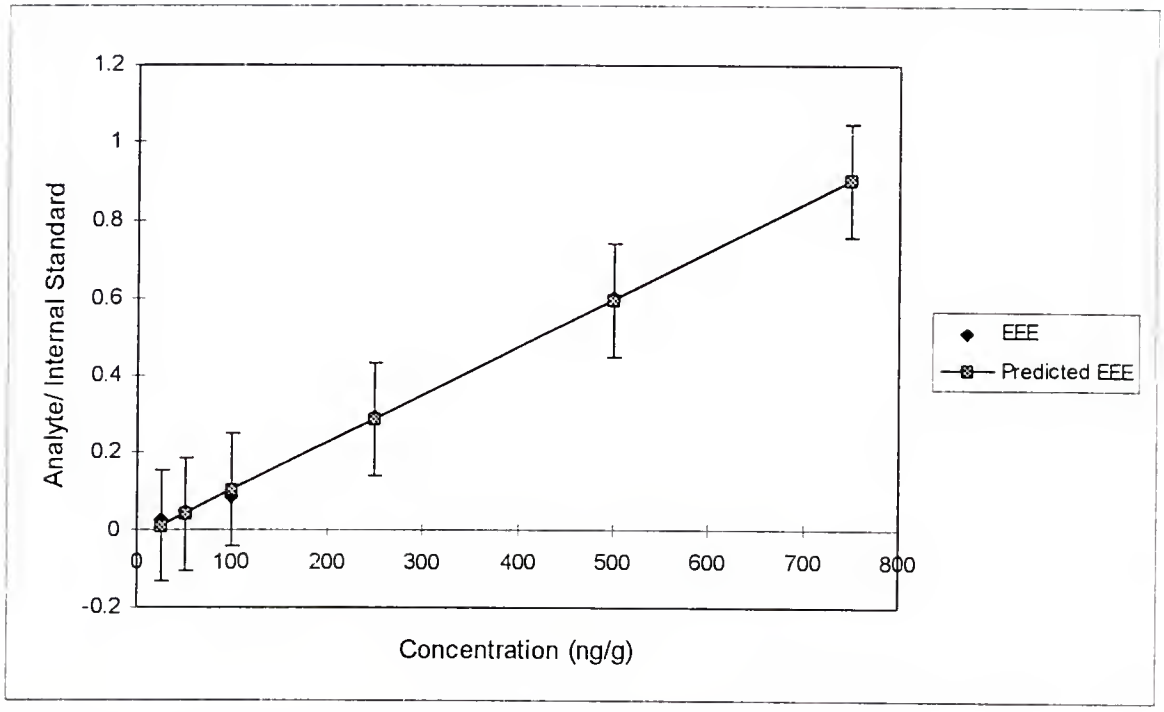
Curve I-13. Benzoylecgonine (BE) in Urine.



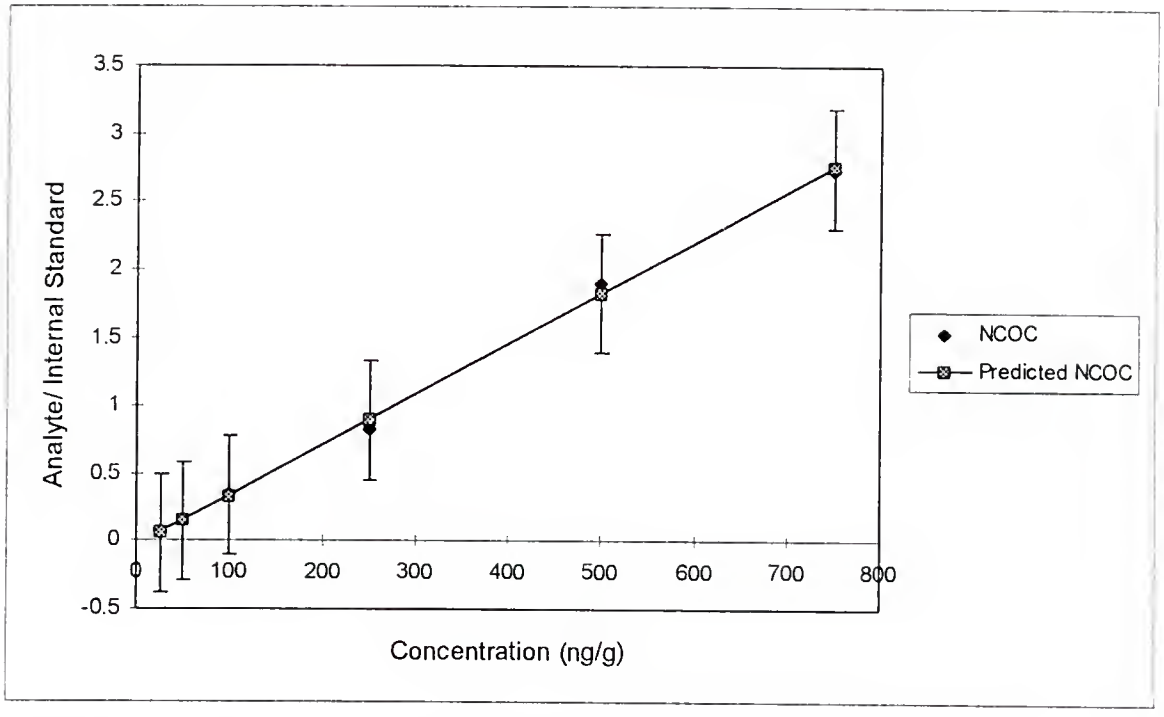
Curve I-14. *m*-Hydroxybenzoylecgonine (MOHBE) in Urine.



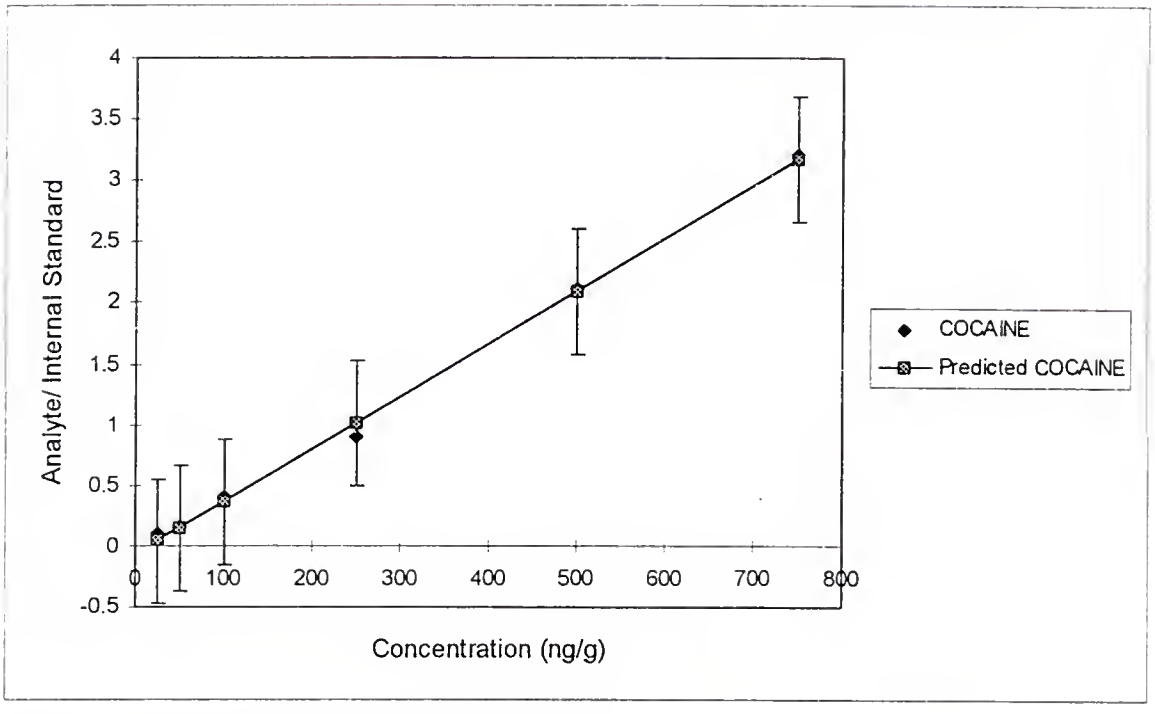
Curve I-15. Ecgonine Methyl Ester (EME) in Meconium.



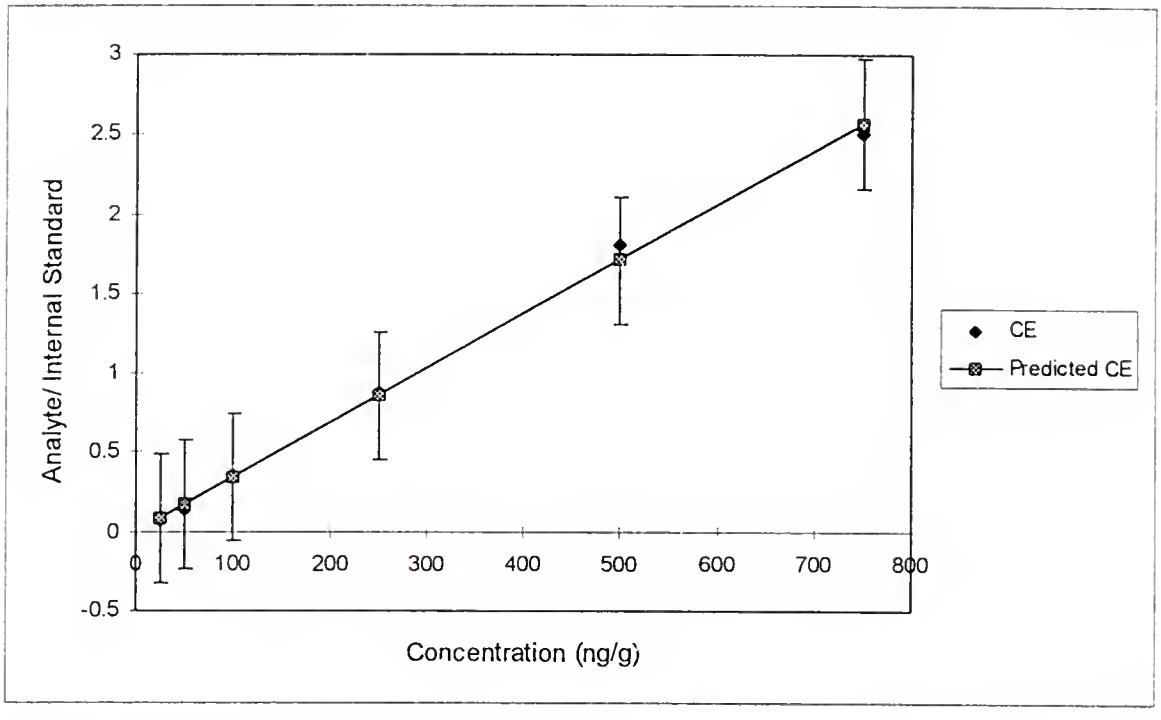
Curve I-16. Ecgonine Ethyl Ester (EEE) in Meconium.



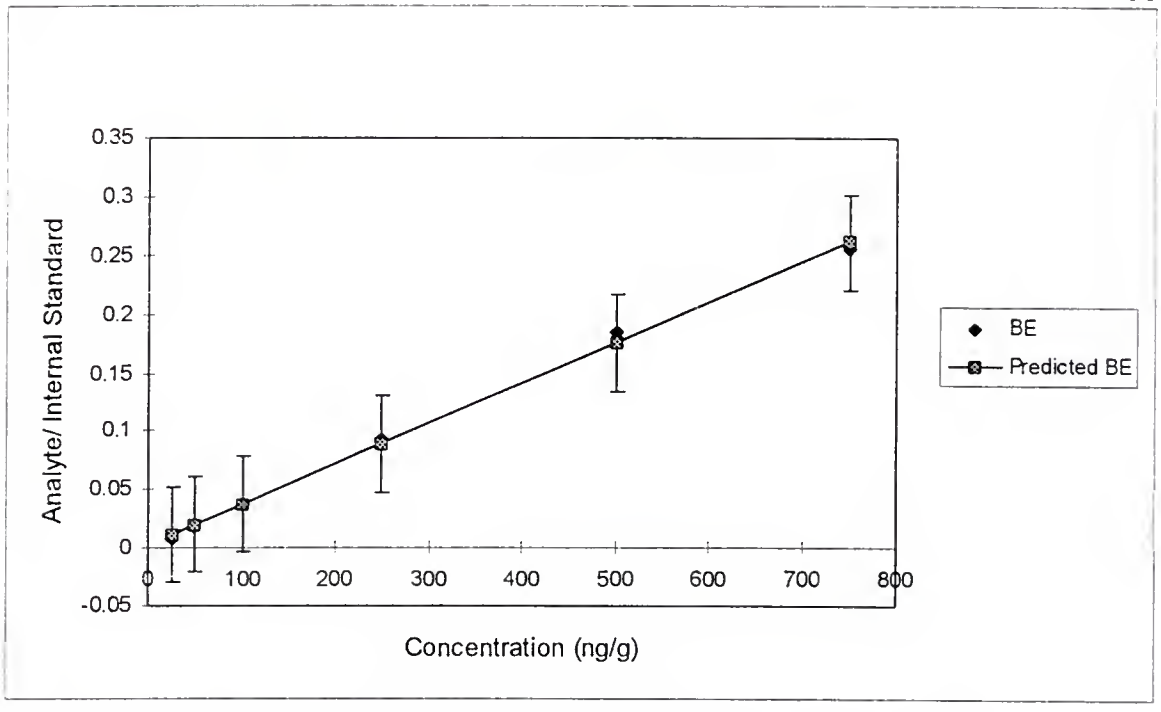
Curve I-17. Norcocaine (NCOC) in Meconium.



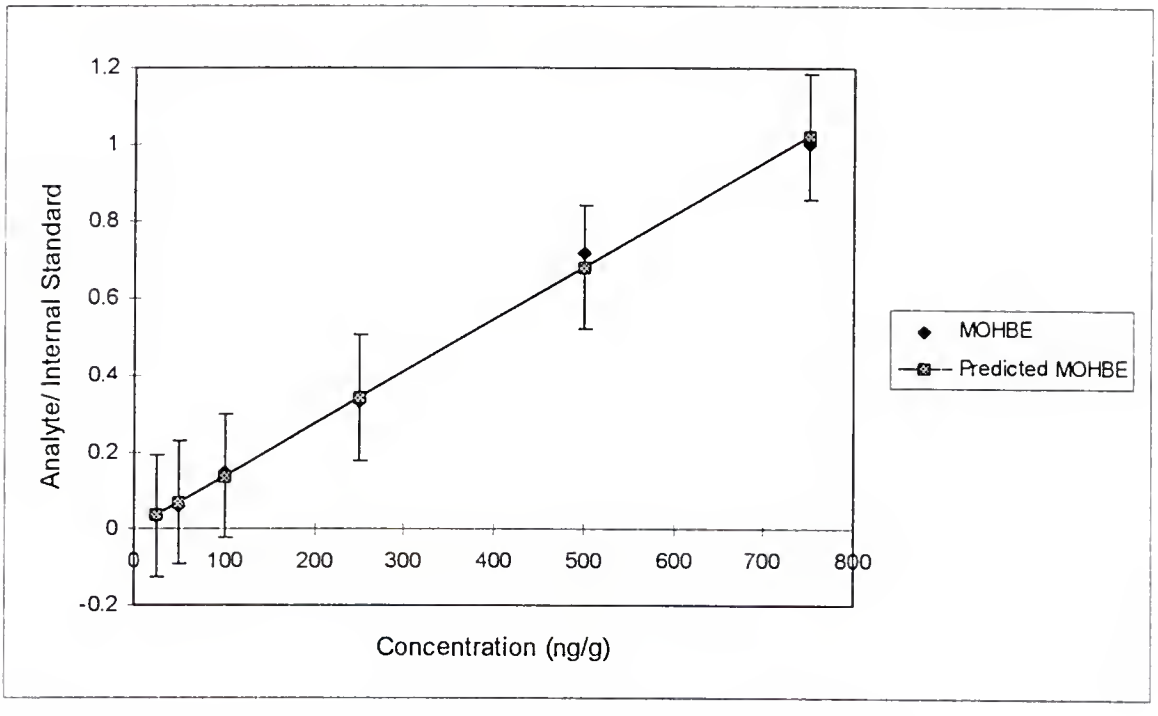
Curve I-18. Cocaine (COC) in Meconium.



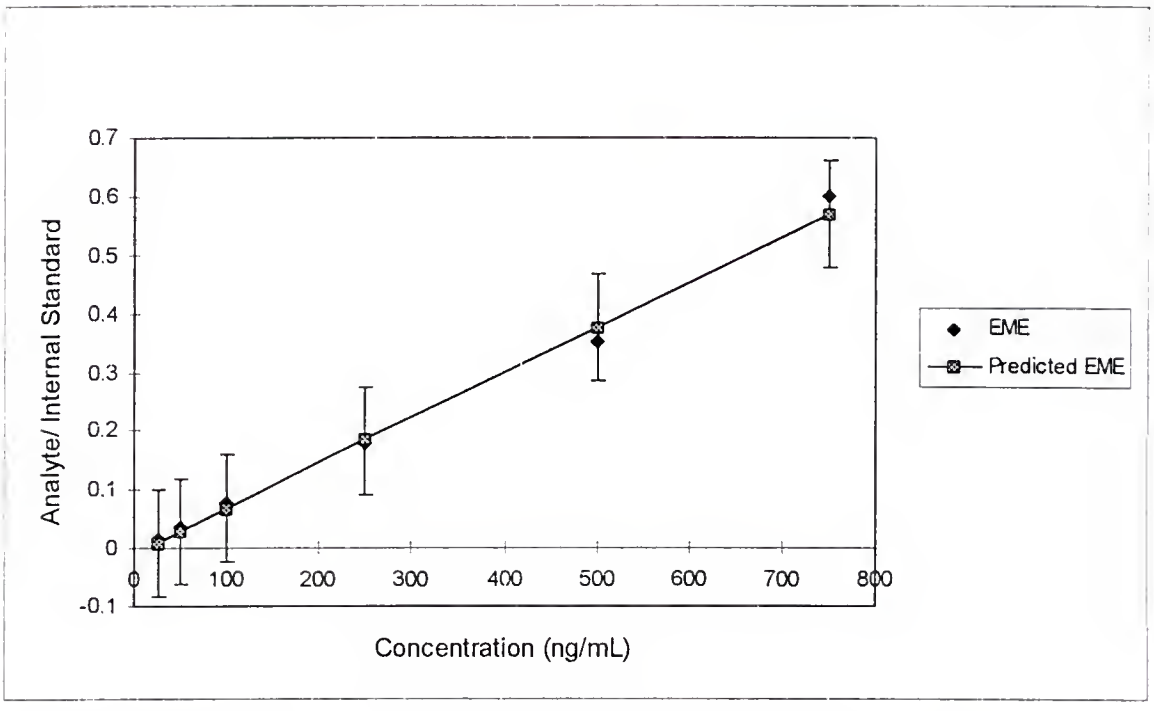
Curve I-19 Cocaethylene (CE) in Meconium.



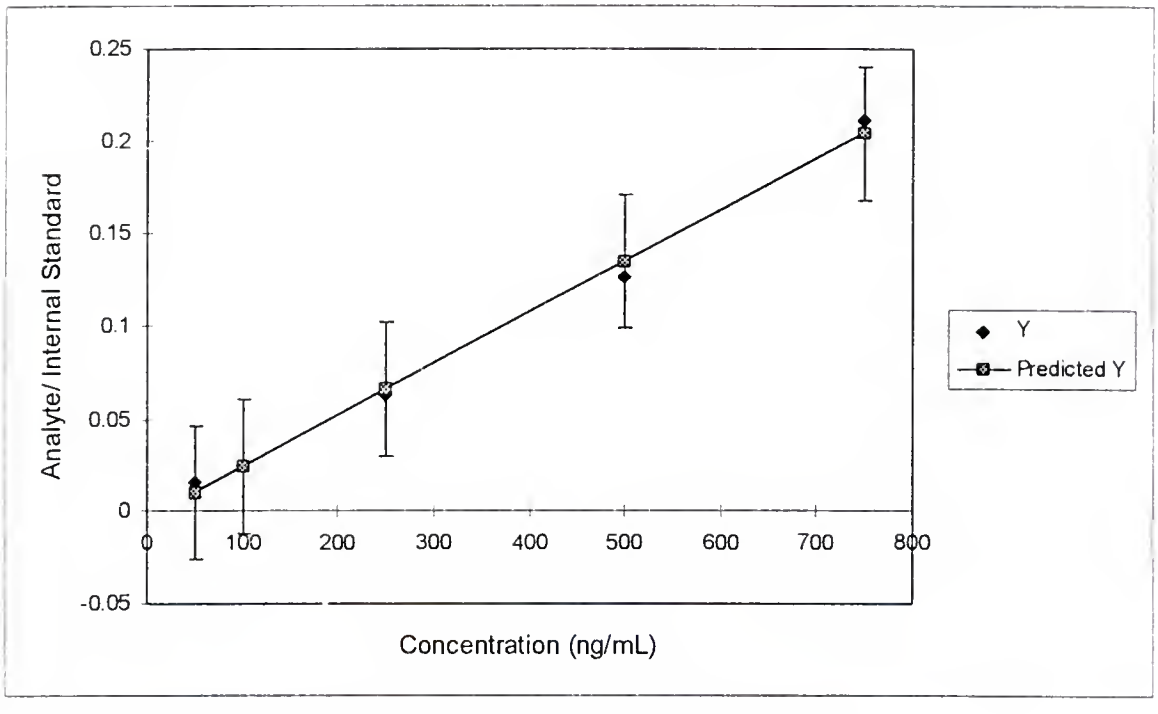
Curve I-20. Benzoylecgonine (BE) in Meconium.



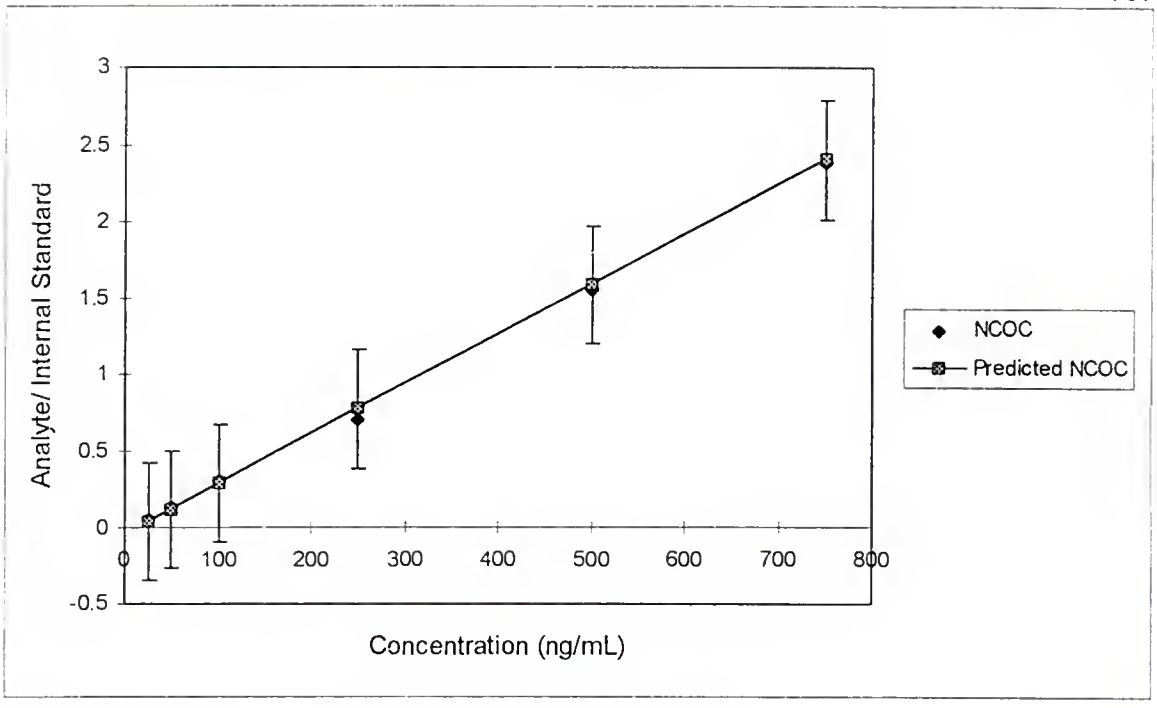
Curve I-21. *m*-Hydroxybenzoylecgonine (MOHBE) in Meconium.



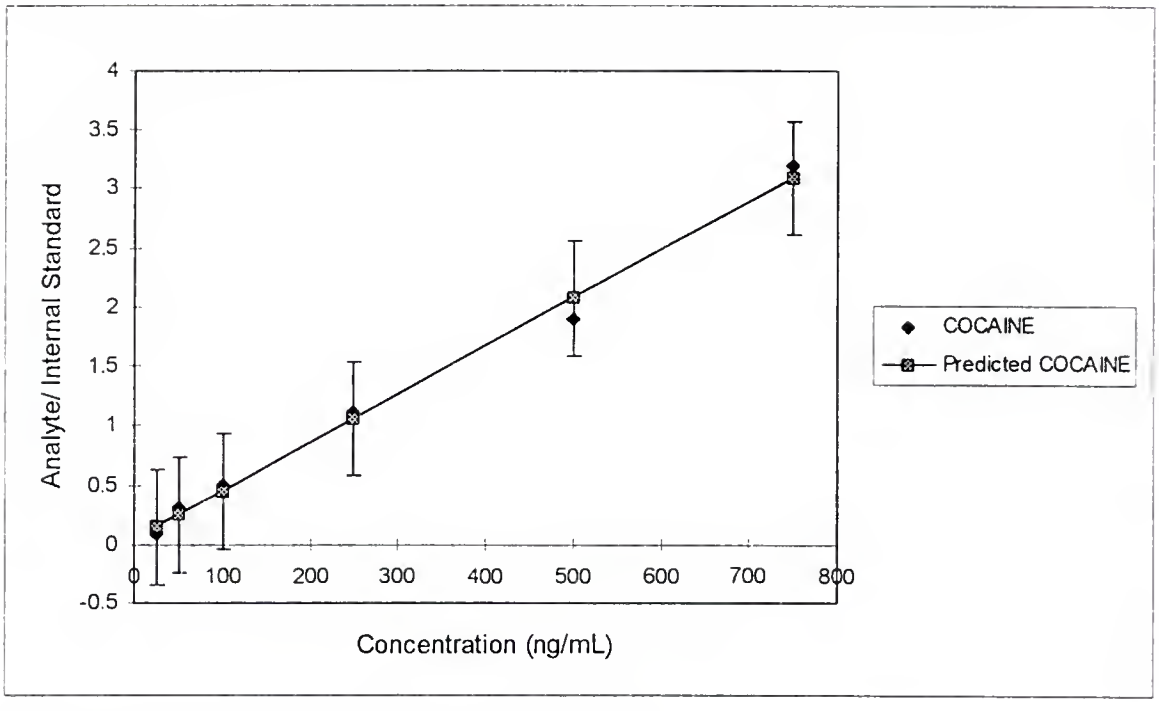
Curve I-22. Ecgonine Methyl Ester (EME) in Amniotic Fluid.



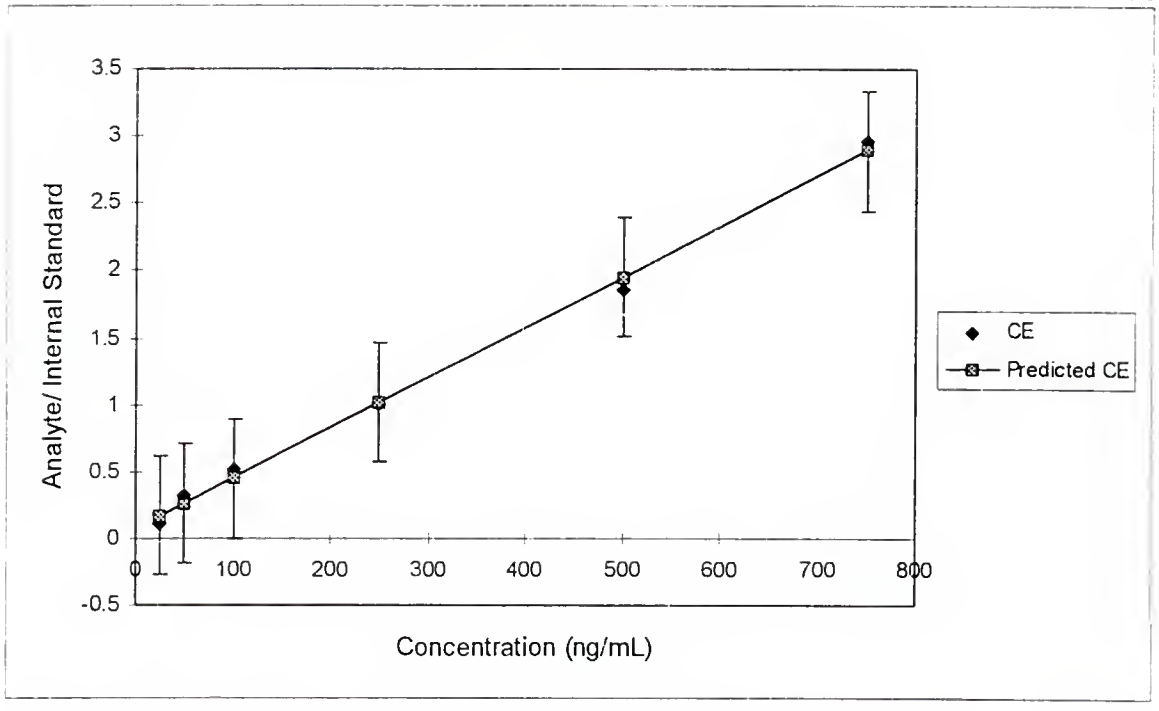
Curve I-23. Ecgonine Ethyl Ester (EEE) in Amniotic Fluid.



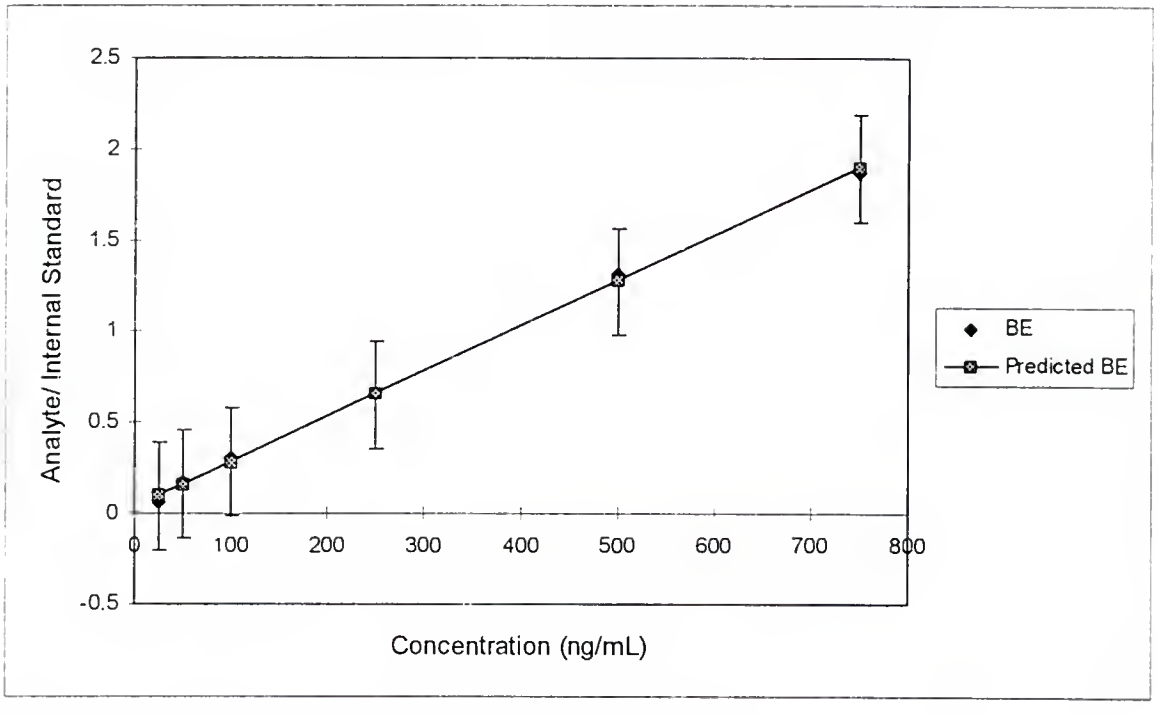
Curve I-24. Norcocaine (NCOC) in Amniotic Fluid.



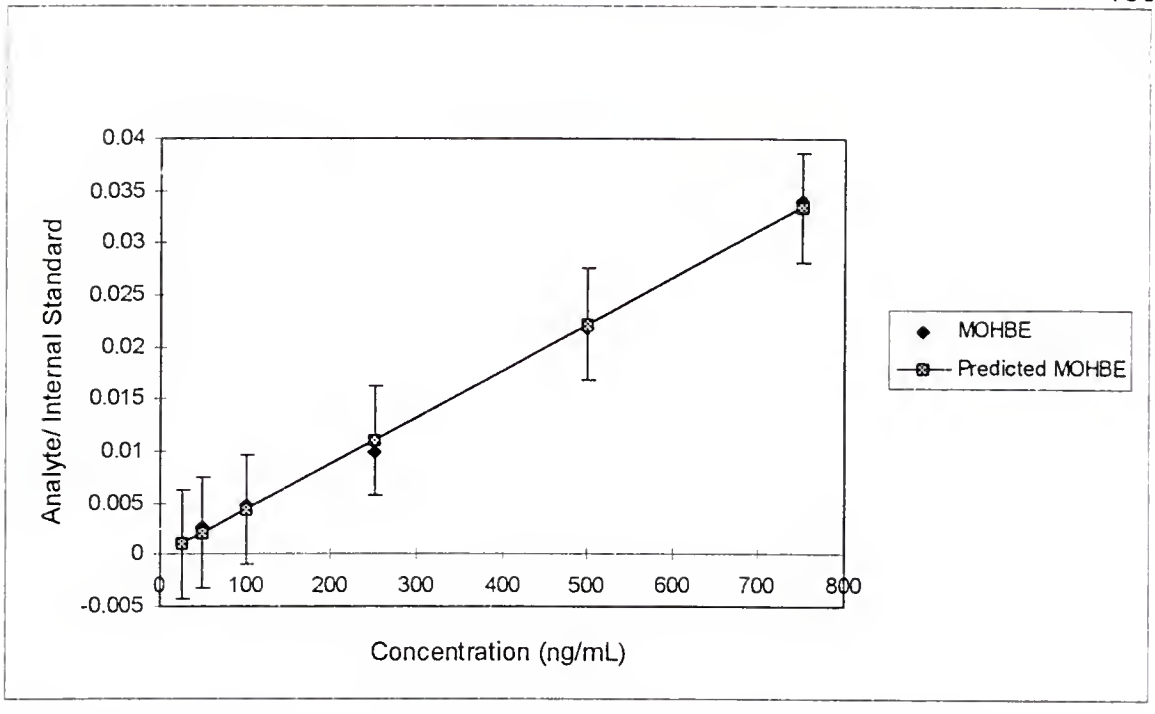
Curve I-25. Cocaine (COC) in Amniotic Fluid.



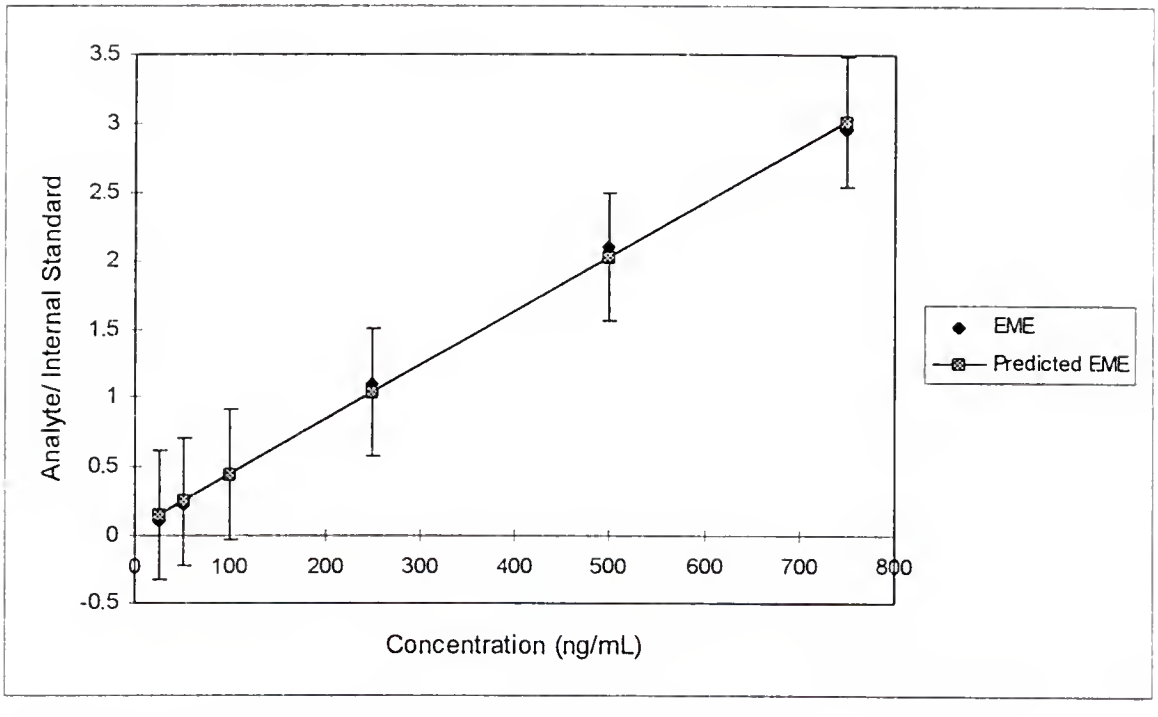
Curve I-26. Cocaethylene (CE) in Amniotic Fluid.



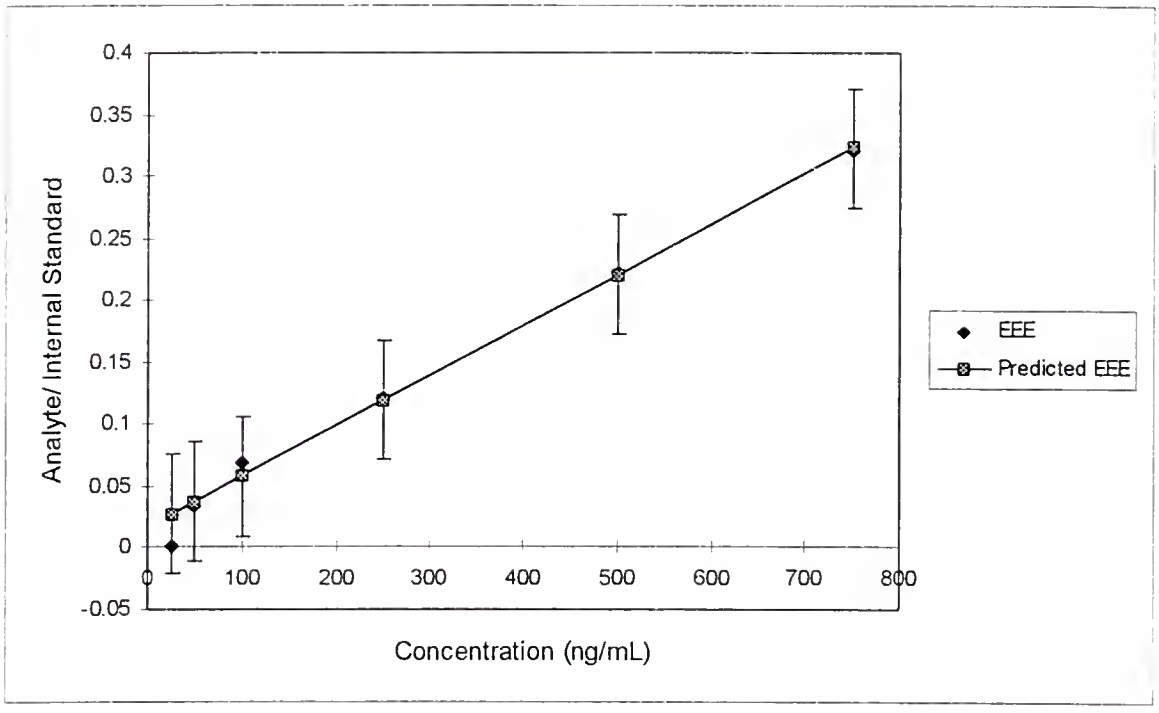
Curve I-27. Benzoylecgonine (BE) in Amniotic Fluid.



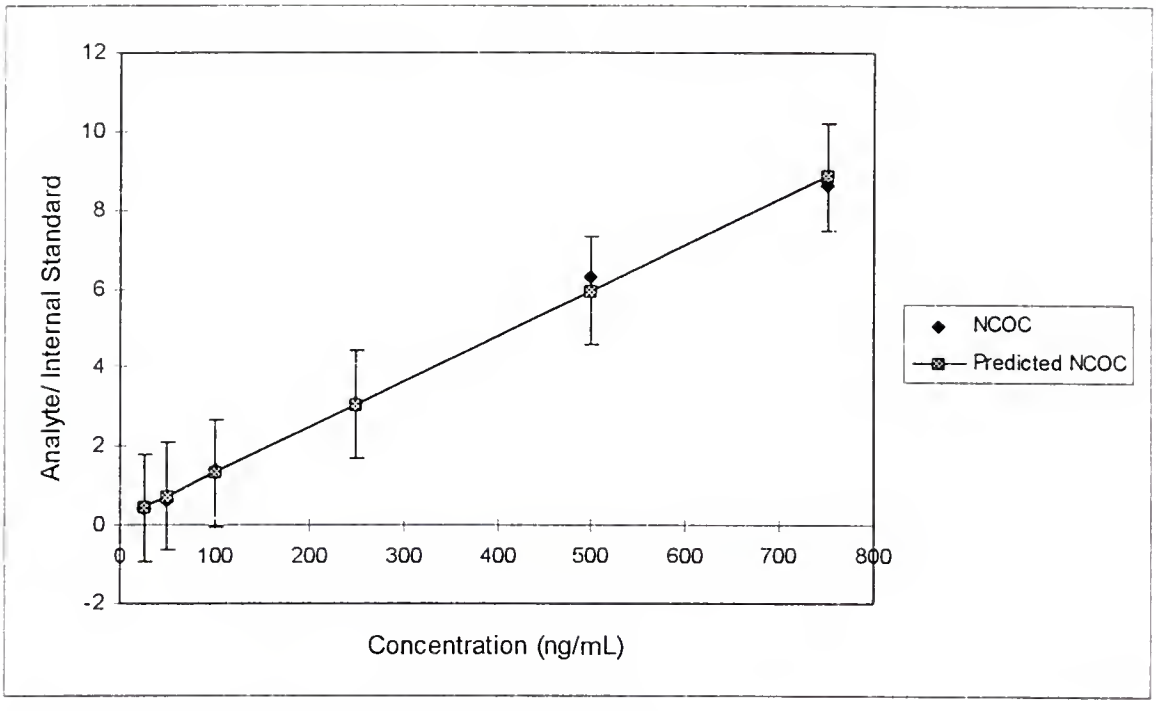
Curve I-28. *m*-Hydroxybenzoylecgonine (MOHBE) in Amniotic Fluid.



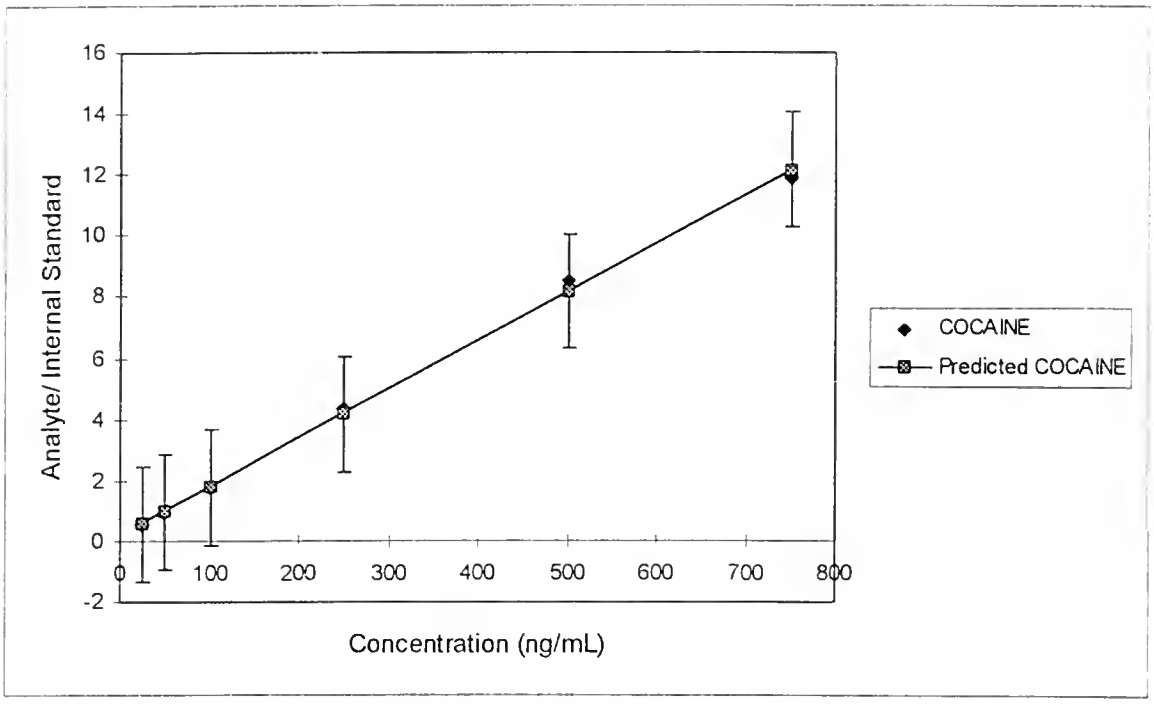
Curve I-29. Ecgonine Methyl Ester (EME) in Colostrum.



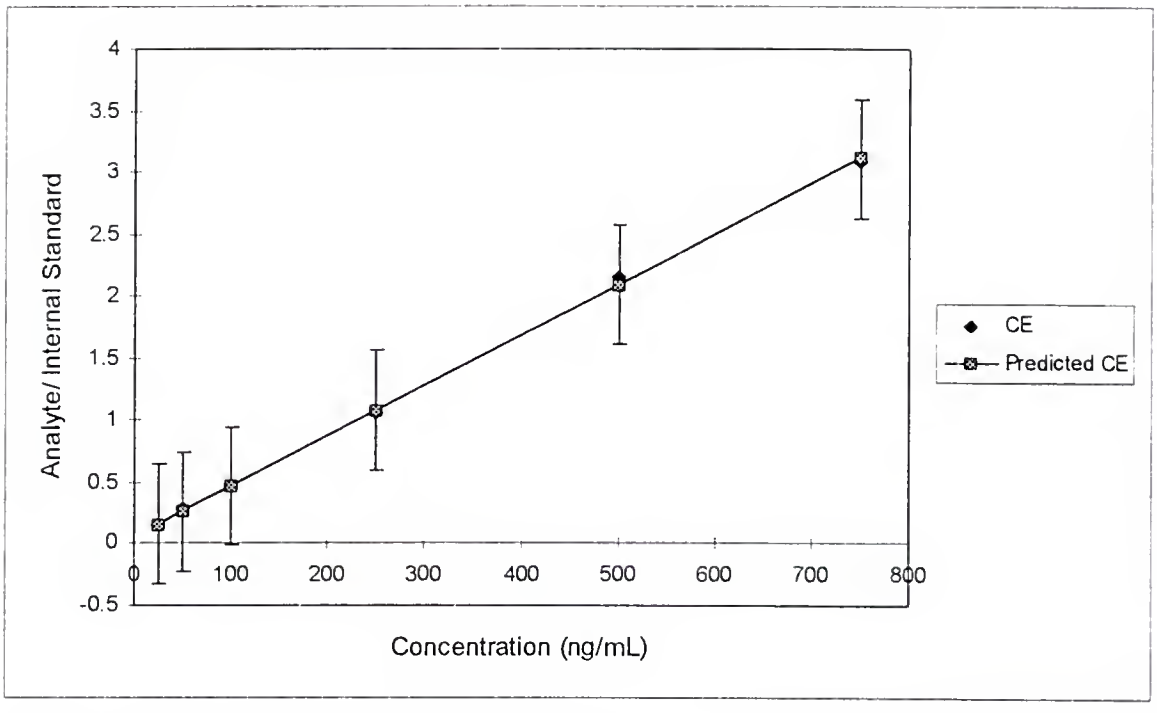
Curve I-30. Ecgonine Ethyl Ester (EEE) in Colostrum.



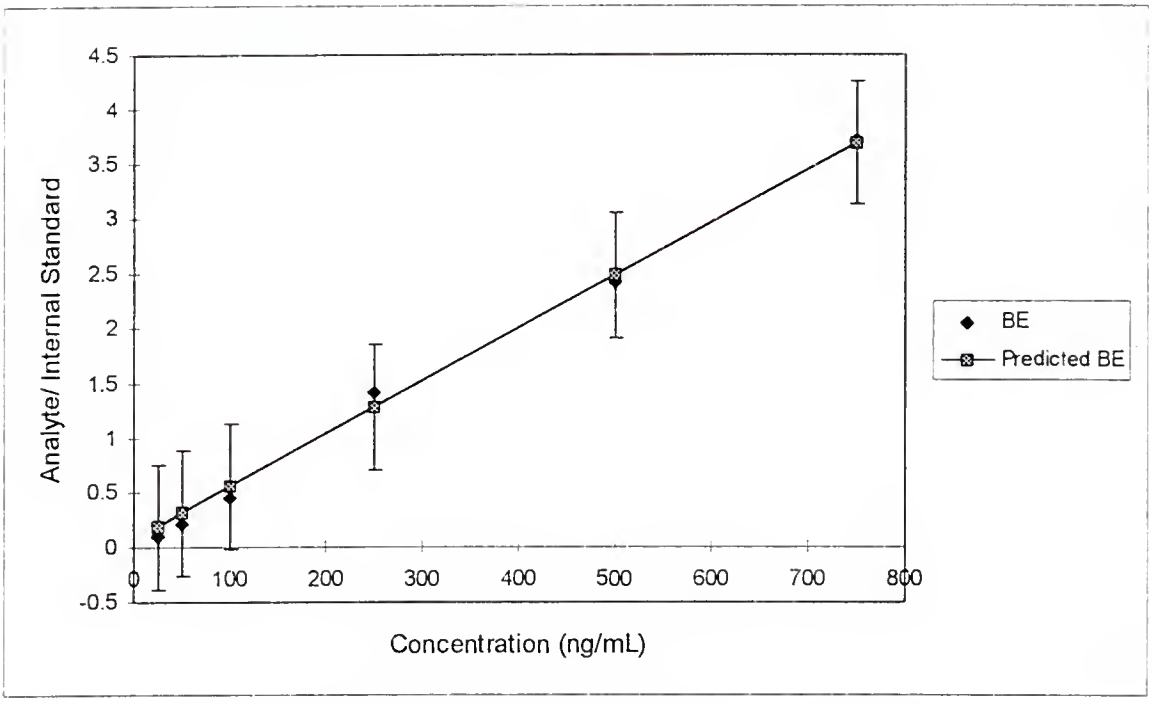
Curve I-31. Norcocaine (NCOC) in Colostrum.



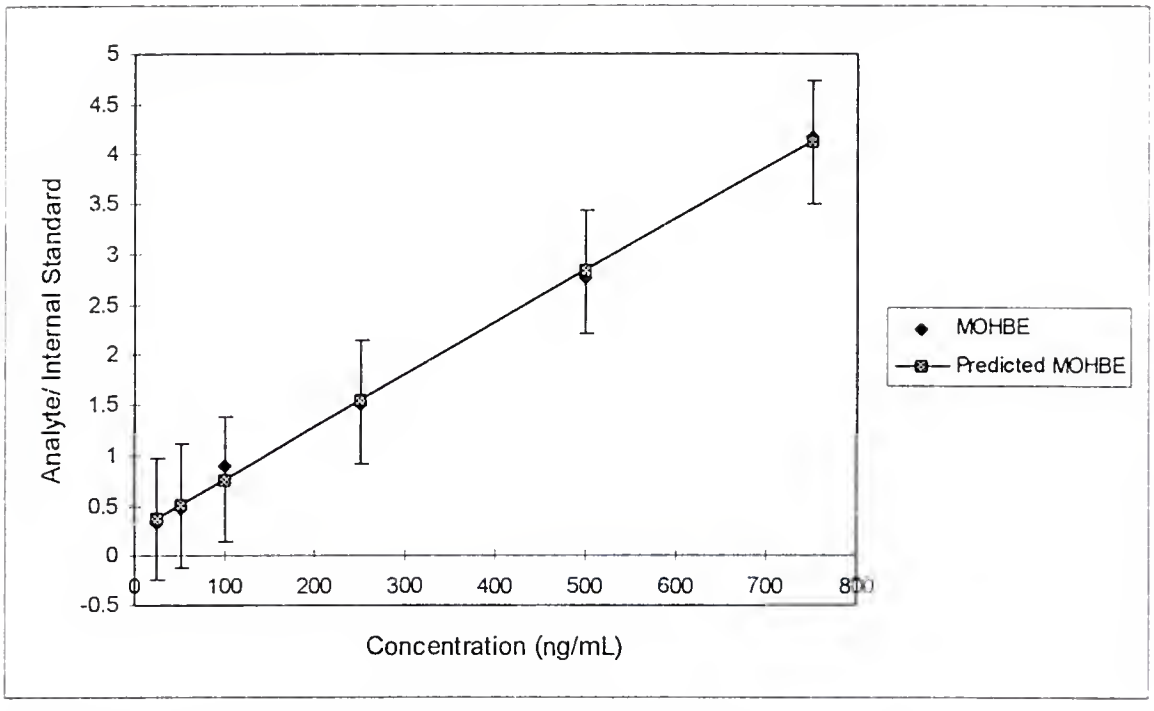
Curve I-32. Cocaine (COC) in Colostrum.



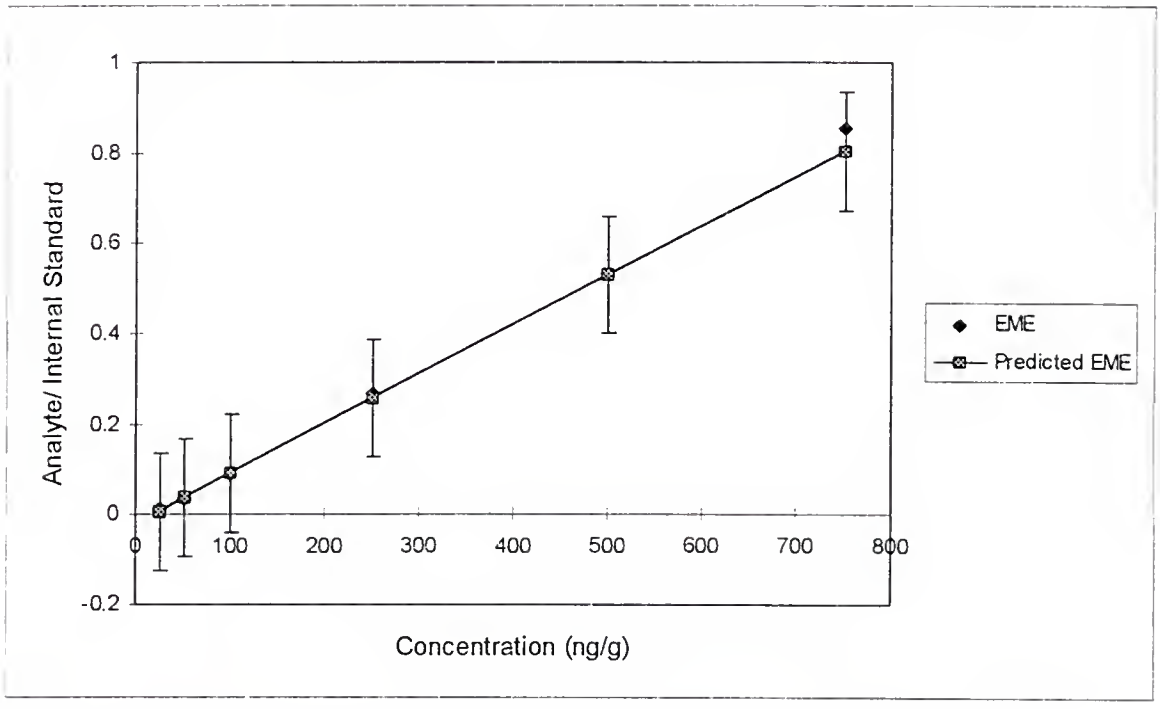
Curve I-33. Cocaethylene (CE) in Colostrum.



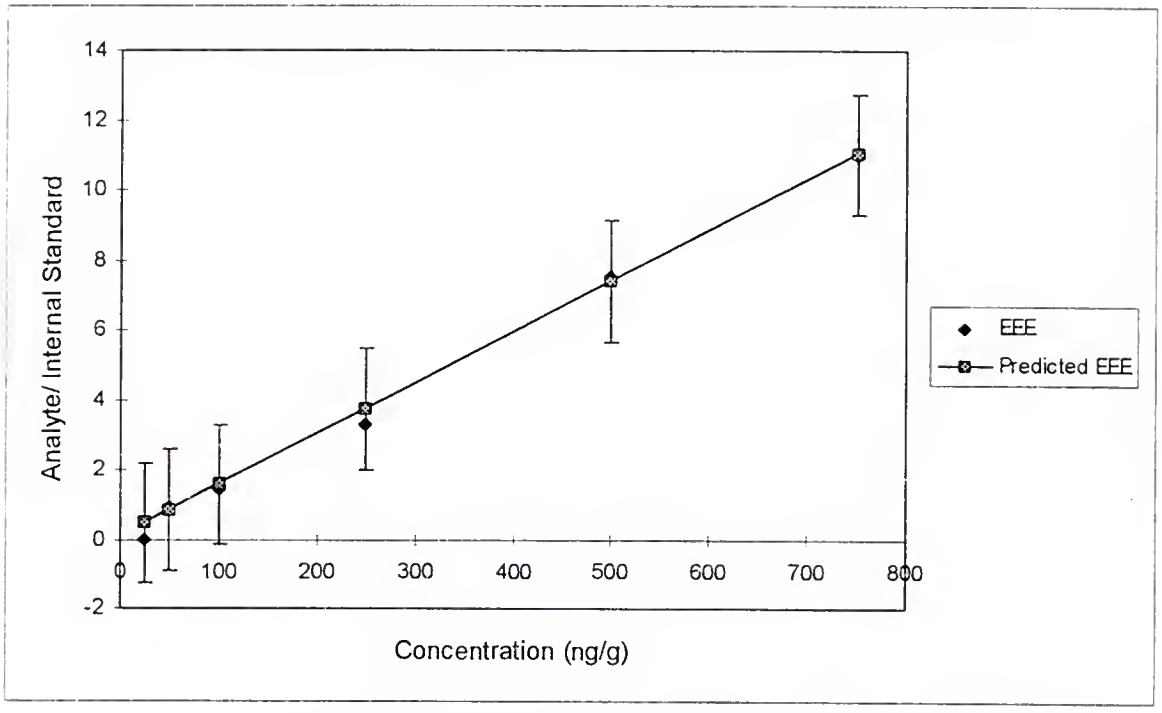
Curve I-34. Benzoylecgonine (BE) in Colostrum.



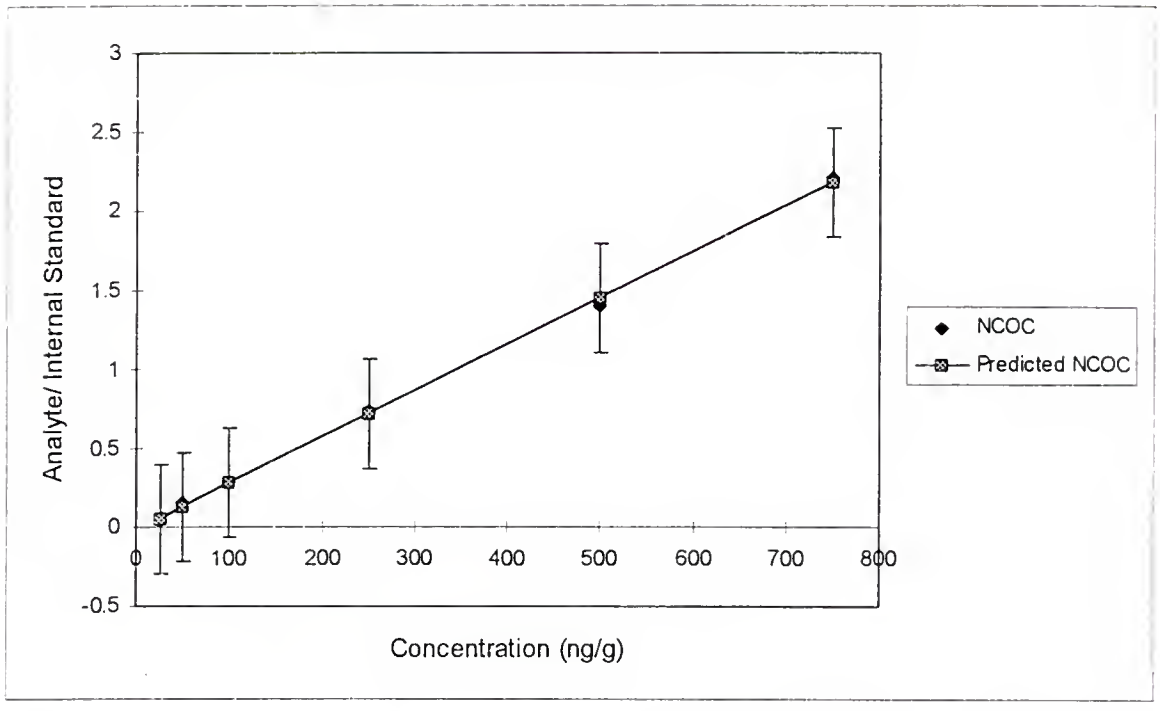
Curve I-35. *m*-Hydroxybenzoylecgonine (MOHBE) in Colostrum.



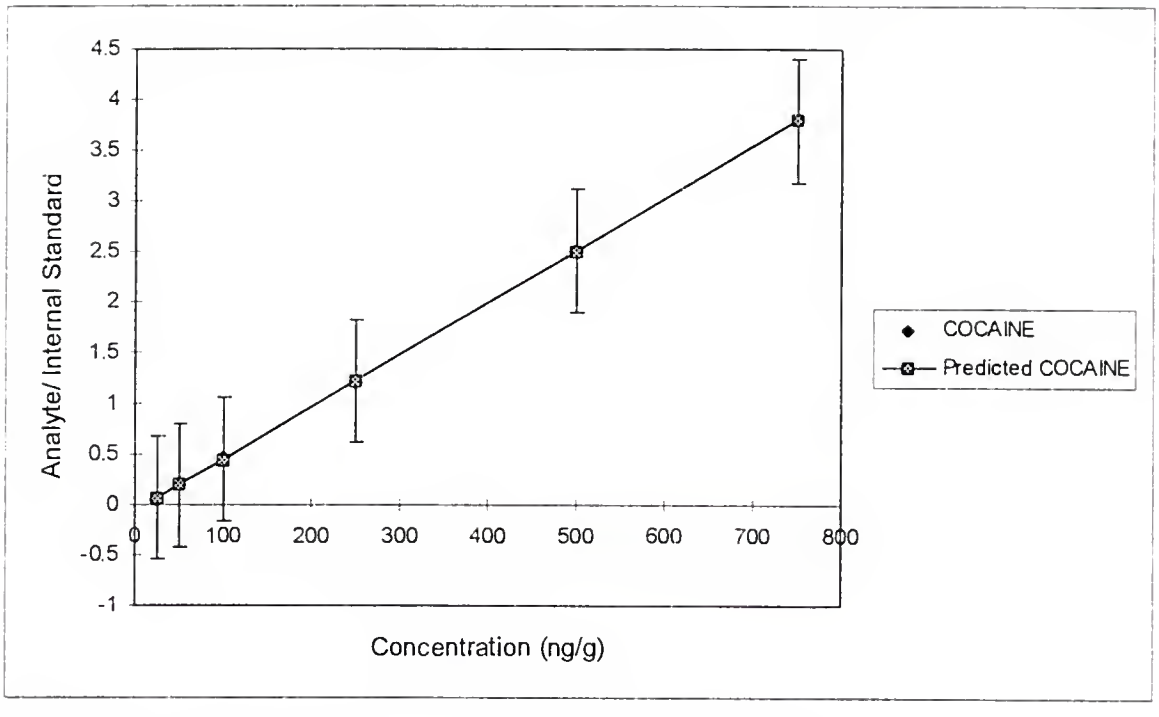
Curve I-36. Ecgonine Methyl Ester (EME) in Umbilical Cord Tissue.



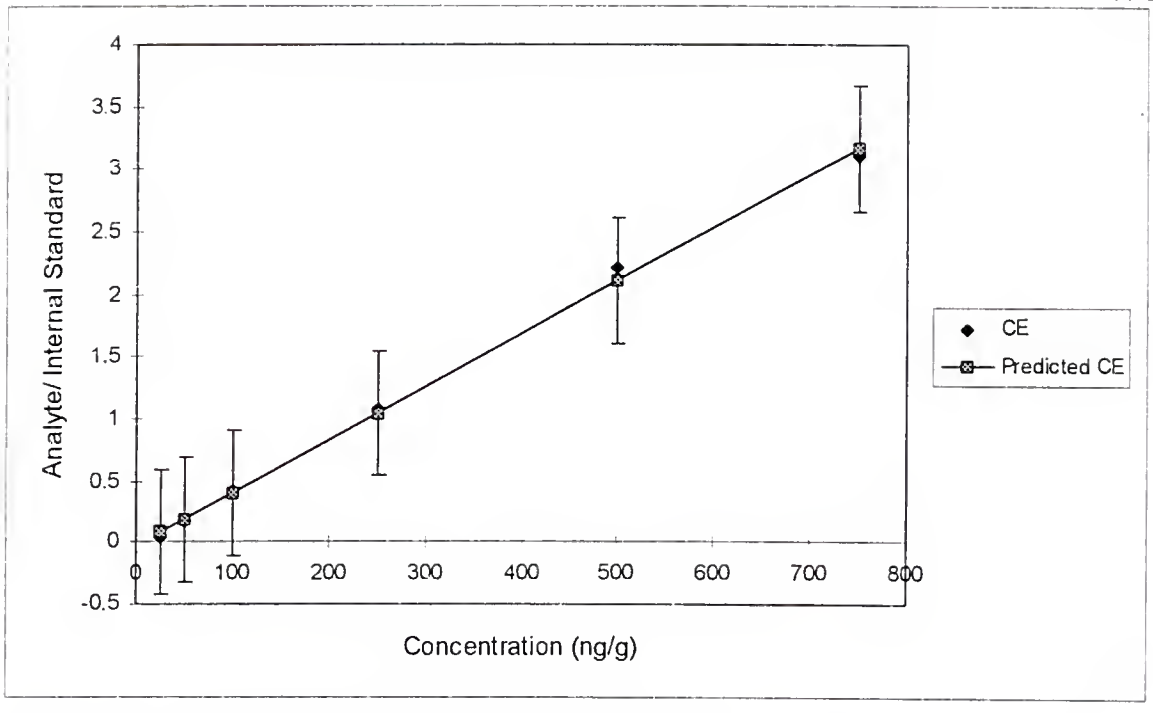
Curve I-37. Ecgonine Ethyl Ester (EEE) in Umbilical Cord Tissue.



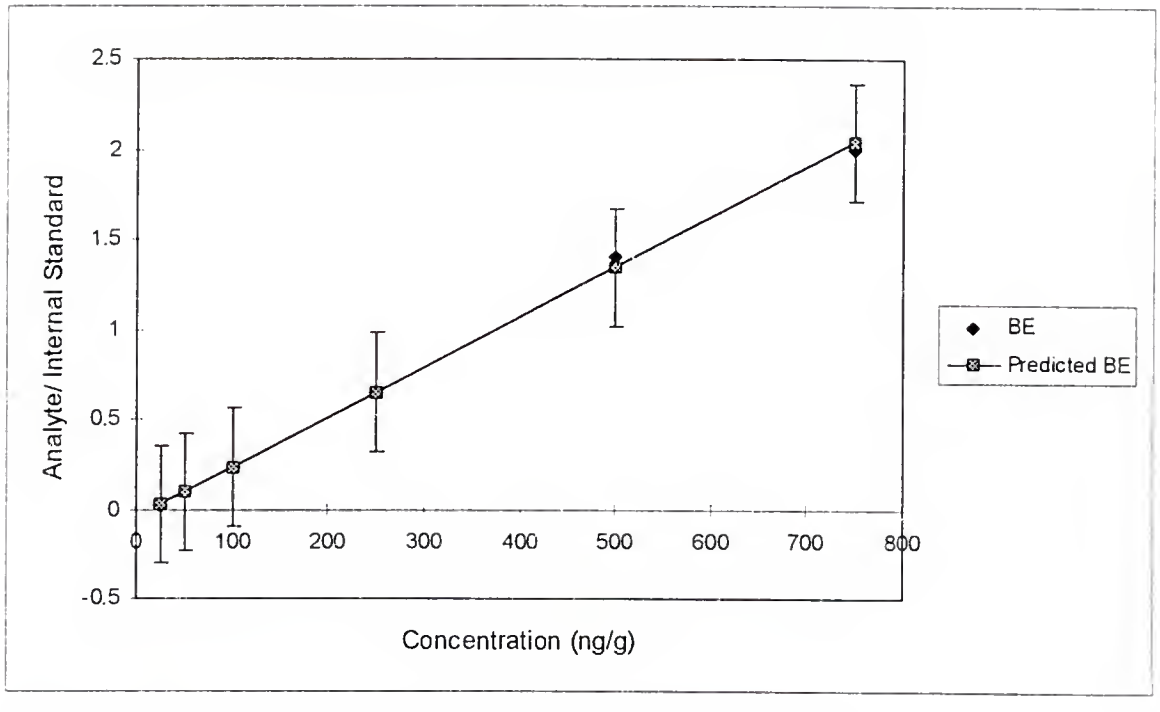
Curve I-38. Norcocaine (NCOC) in Umbilical Cord Tissue.



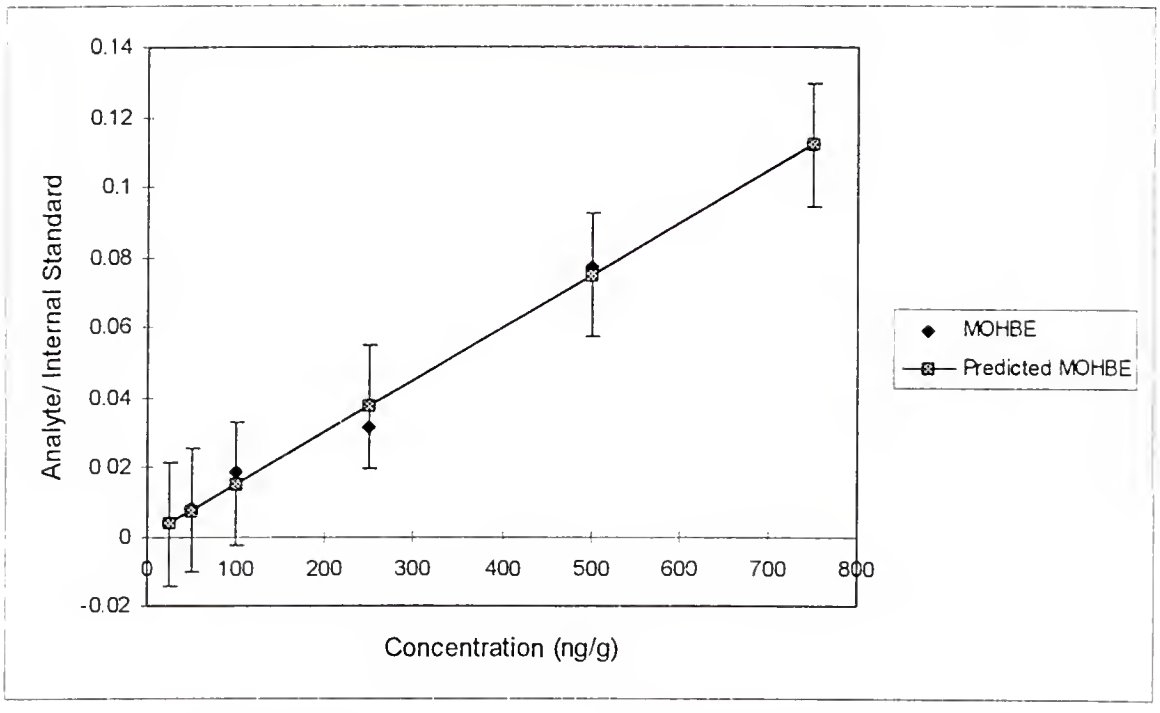
Curve I-39. Cocaine (COC) in Umbilical Cord Tissue.



Curve I-40. Cocaethylene (CE) in Umbilical Cord Tissue.



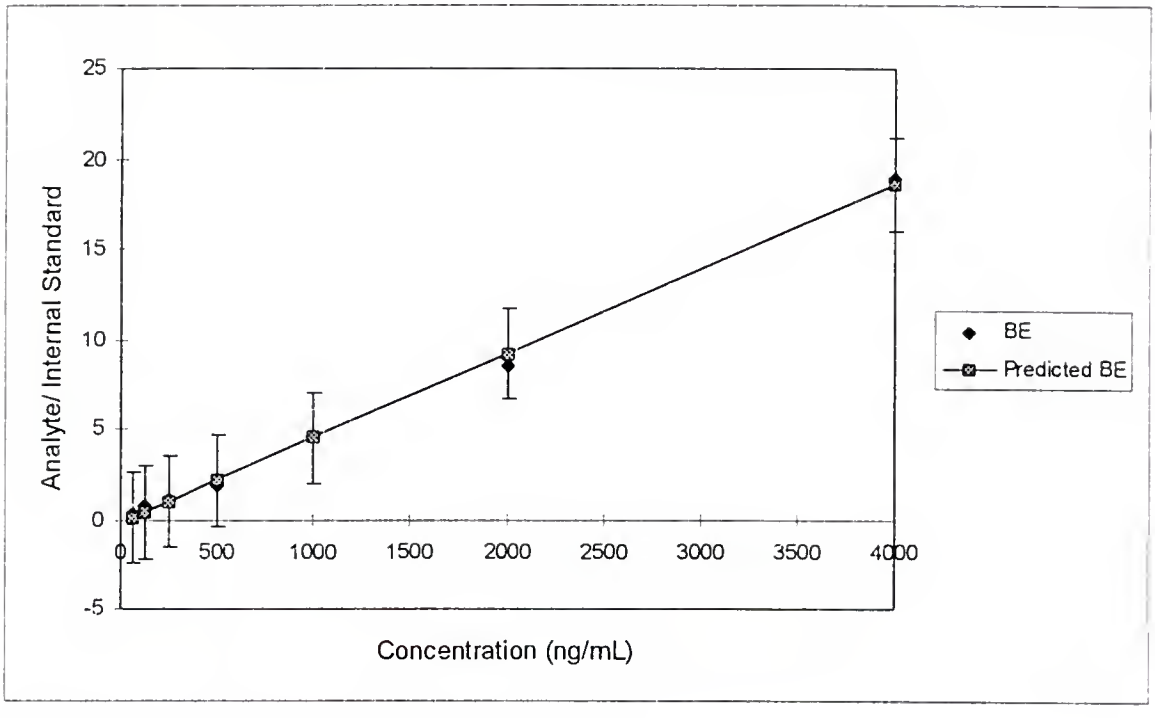
Curve I-41. Benzoylecgonine (BE) in Umbilical Cord Tissue.



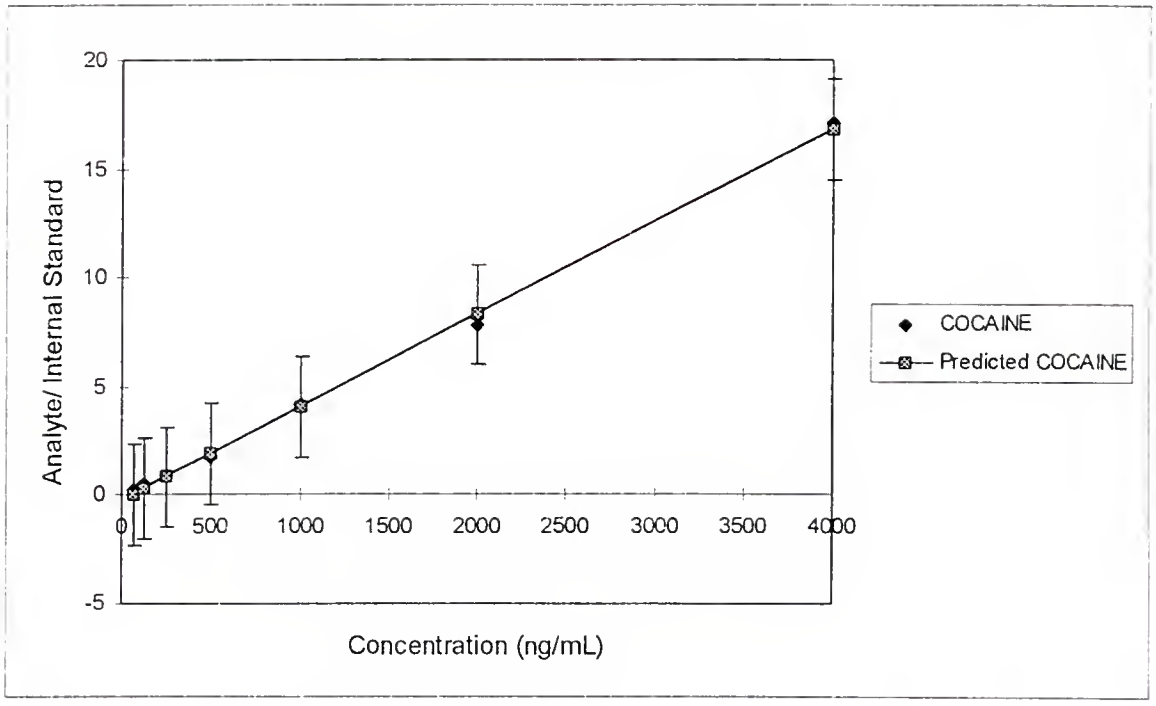
Curve I-42. *m*-Hydroxybenzoylecgonine (MOHBE) in Umbilical Cord Tissue.

APPENDIX II: HPLC CALIBRATION CURVES

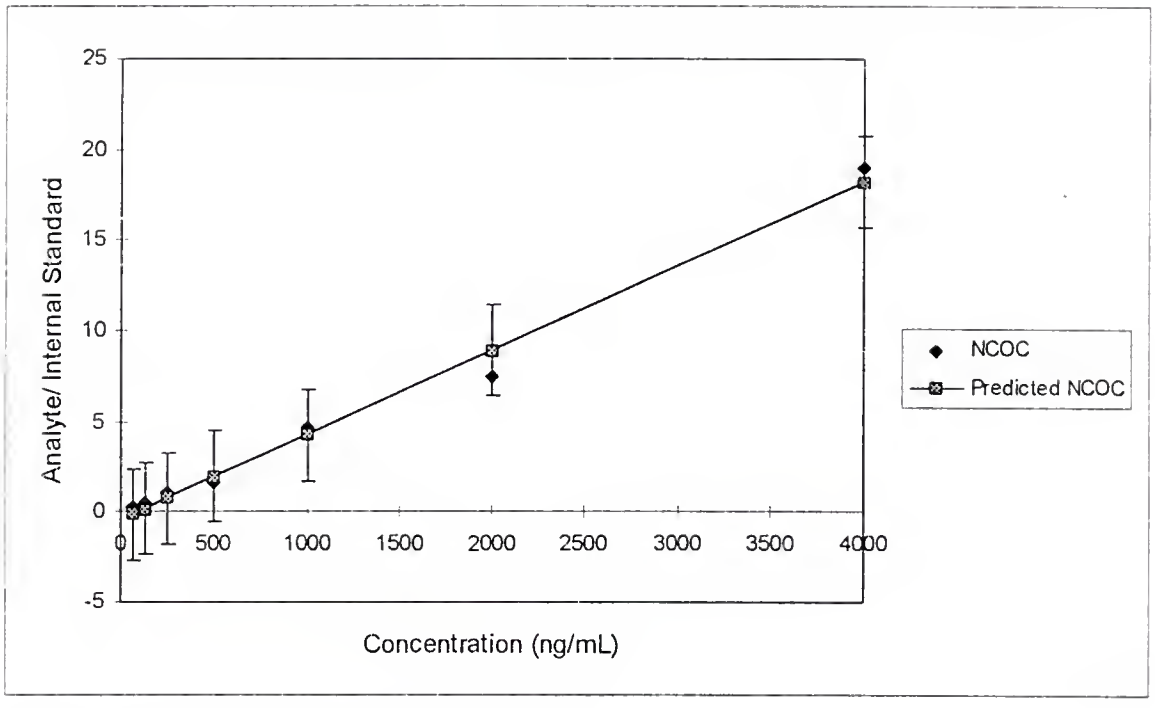
Representative HPLC calibration curves for benzoylecgonine, cocaine, norcocaine, and cocaethylene, in urine and meconium. Curves are plotted as ratio of analyte quantitation ion to internal standard quantitation ion versus concentration. Curves include actual points, regression analysis predicted points and standard error bars. In linear curves, all points will fall within the standard error area.



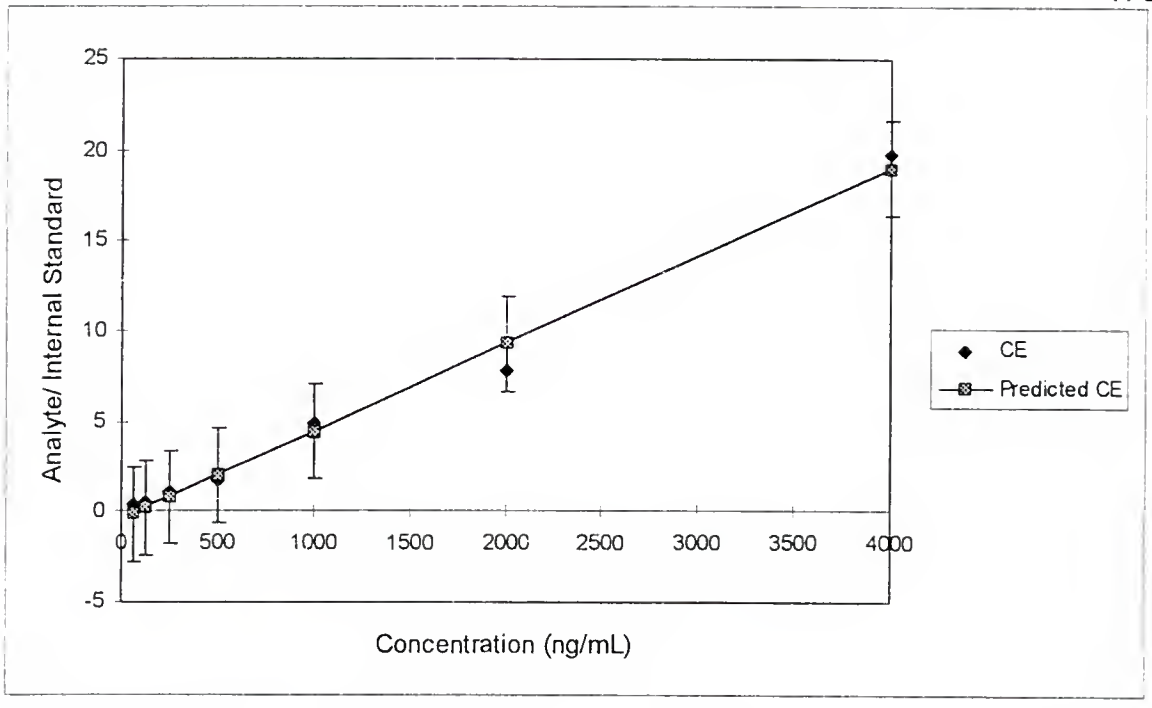
Curve II-1. Benzoylecgonine (BE) in Urine.



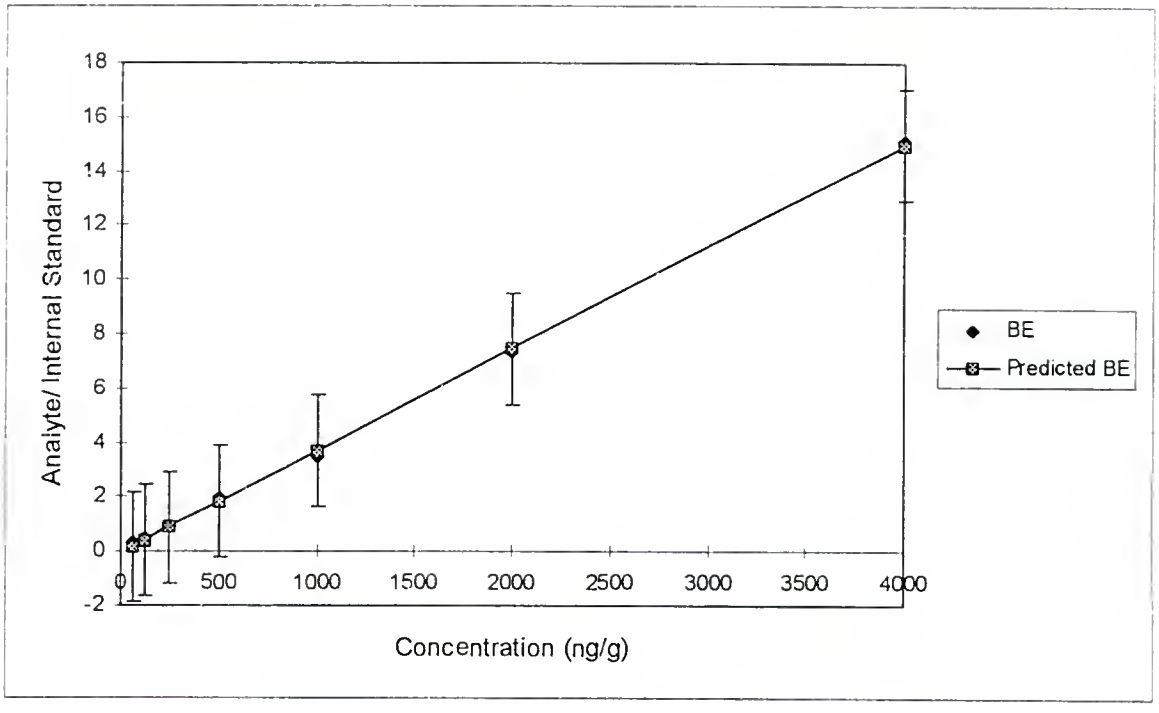
Curve II-2. Cocaine (COC) in Urine.



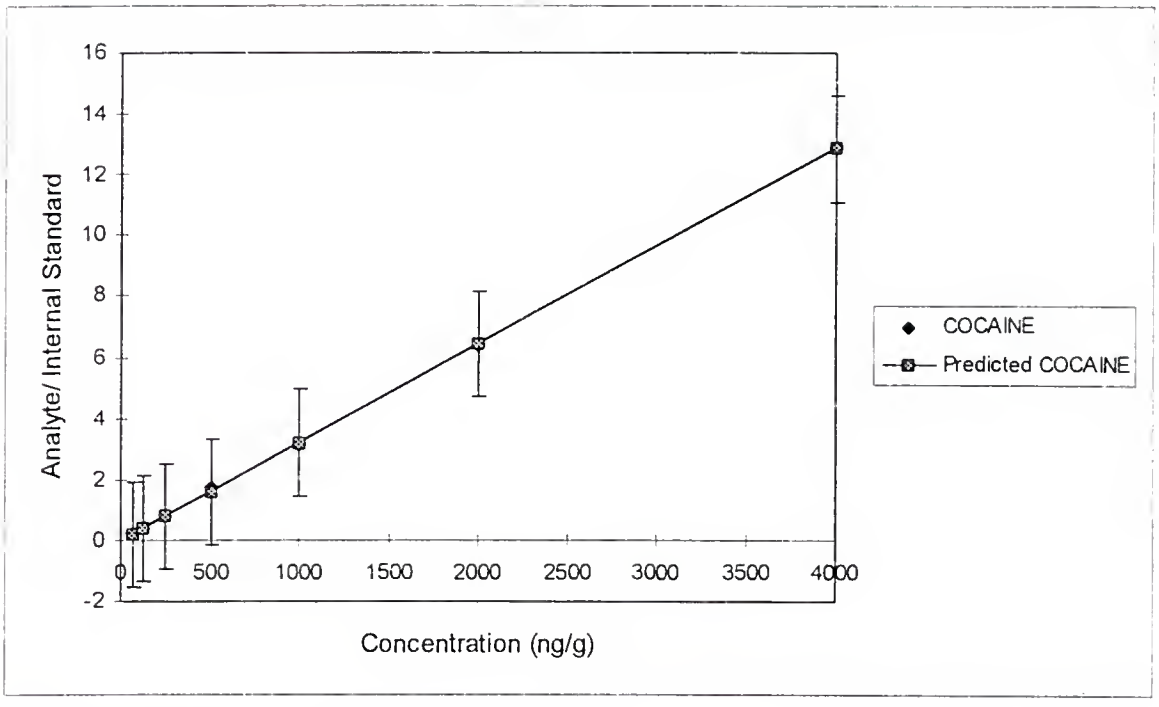
Curve II-3. Norcocaine (NCOC) in Urine.



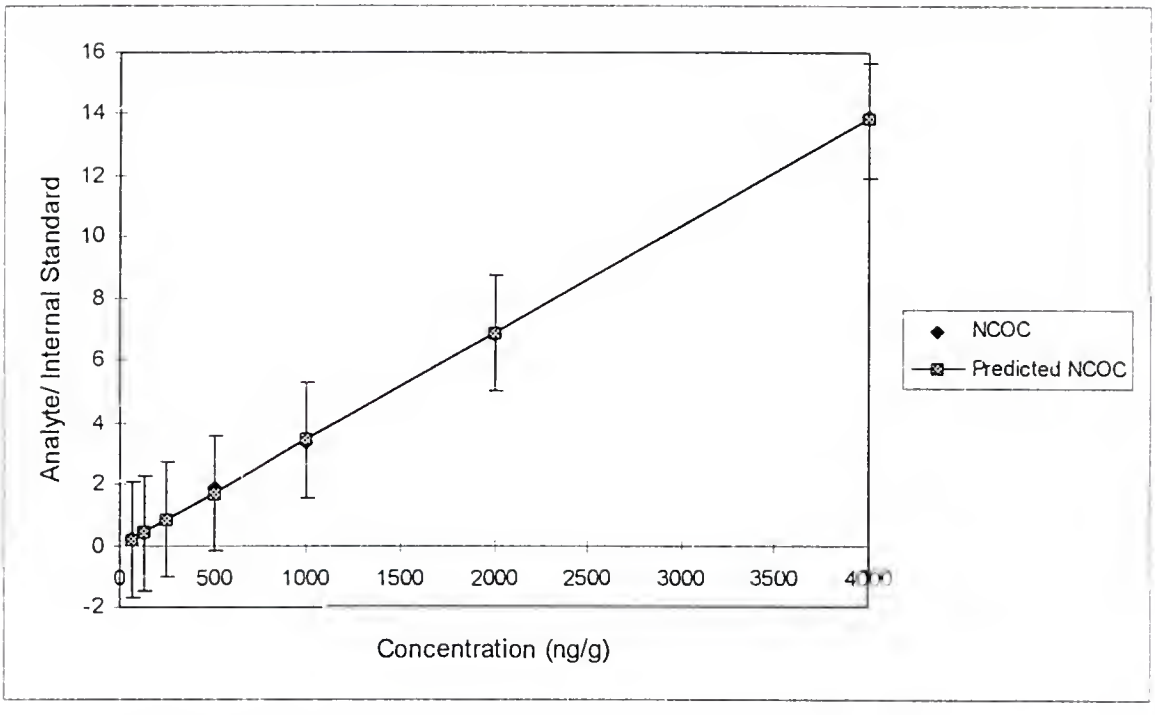
Curve II-4. Cocaethylene (CE) in Urine.



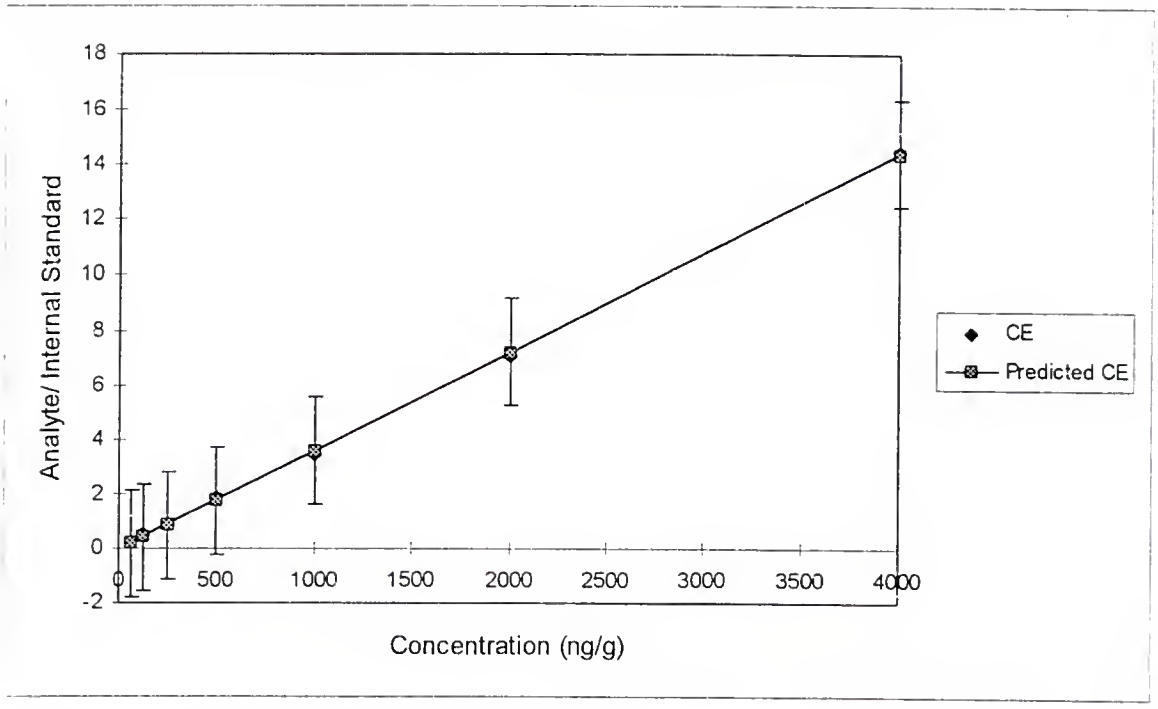
Curve II-5. Benzoylecgonine (BE) in Meconium.



Curve II-6. Cocaine (COC) in Meconium.



Curve II-7. Norcocaine (NCOC) in Meconium



Curve II-8. Cocaethylene (CE) in Meconium.

REFERENCES

1. M.J. Ellenhorn and D.G. Barceloux, ed. Medical Toxicology, Diagnosis and Treatment of Human Poisoning. Elsevier Science Publishing, Inc. New York. p644-661 (1988).

2. Weiss, S.M. Mirin, and R.L. Bartel. Cocaine, 2nd Ed. American Psychiatric Press, Inc. Washington D.C. 1-70 (1994).

3. Self, W.J. Barnhart, D.A. Lehman, and E.J. Nestler. Opposite modulation of cocaine-seeking behavior by D_1 and D_2-like dopamine receptor agonists. Science. 271: 1586-1589 (1996).

4. T.L. Hawley, T.G. Halle, R.E. Drasin, and N.G. Thomas. Children of addicted mothers: effects of the 'crack epidemic' on the caregiving environment and the development of preschoolers. American Orthopsychiatric Association, Inc. 164-379 (1995).

5. SAMHSA. National household survey on drug abuse: population estimates 1992. U.S. Department of Health and Human Services, Baltimore. 1993.

6. S.B. Karch, and M.E. Billingham. The pathology and etiology of cocaine-induced heart disease. Archives of Pathology and Laboratory Medicine. 112: 225-230 (1988).

7. M.S. Gold. Cocaine, Drugs of Abuse: A Comprehensive Series for Clinicians. Vol. 3. Plenum Publishing Corp. New York. p1-79 (1993).

8. H.C. Mofenson and T.R. Caraccio. Cocaine. Pediatrics Annals. 16: 864-874 (1987).

9. G. Burkett, S.Y. Yasin. D. Palow, L. LaVoie, and M. Martinez. Paterns of cocaine binging: effect on pregnancy. American Journal of Obstetrics and Gynecology. 171: 372-379 (1994).

10. F.D. Eyler, M. Behnke, M. Conlon, N.S. Woods, and B. Frentzen. Prenatal cocaine use: a comparison of neonates matched on maternal risk factors. Neurotoxicology and Teratology. 16: 81-87 (1994).

11. M. Battin, S. Albersheim, and D. Newman. Congenital genitourinary tract abnormalities following cocaine exposure in utero. American Journal of Perinatology. 12: 425-428 (1995).

12. J.G. Ray. Lues-Lues: maternal and fetal considerations of syphilis. Obstetrical and Gynecological Survey. 50: 845-850 (1995).

13. C. Moore and A. Negrusz. Drugs of abuse in meconium. Forensic Science Review. 7: 104-118 (1995).

14. G. Das. Cocaine abuse in North America: a milestone in history. Journal of Clinical Pharmacology. 33: 296-310 (1993).

15. M.G. Ripple, B.A. Goldberger, Y.H. Caplan, M.G. Blitzer, and S. Schwartz. Detection of cocaine and its metabolites in human amniotic fluid. Journal of Analytical Toxicology. 16:328-331 (1992).

16. A.M. Dusick, R.D. Covert, M.D. Schreiber, G.T. Yee, S.P. Browne, C.M. Moore, and I.R. Tebbett. Risk of intercranial hemorrhage and other adverse outcomes after cocaine exposure in a cohort of 323 very low birth weight infants. J. Pediatr. 122:438-45 (1993).

17. M.W. Fishman. Behavioral pharmacology of cocaine. Journal of Clinical Psychiatry. 49: (Suppl) 7-10 (1988).

18. P.I. Jatlow. Drug of abuse profile: cocaine. Clinical Chemistry. 33: 66B-71B (1987).

19. P.I. Jatlow. Cocaine: analysis, pharmacokinetics, and metabolic disposition. The Yale Journal of Biology and Medicine. 61:105-113 (1988).

20. F.H. Gawin. Chronic neuropharmacology of cocaine: progress in pharmacotherapy. Journal of Clinical Psychiatry. 49: (Suppl) 11-16 (1988).

21. S.E. Krug. Cocaine abuse: historical, epidemiologic, and clinical perspectives for pediatricians. Advances in Pediatrics. 36: 369-406 (1989).

22. C. Ashely and R.A. Hitzeman. Pharmacology of cocaine. In: Cocaine in the Brain. N.D. Volkow and A.C. Swain, eds. Rutgers University Press, New Brunswick,N.J. 33-108 (1990).

23. M. Gossop, P. Griffiths, B. Powis, and J. Strang. Cocaine: patterns of use, route of administration, and severity of dependence. British Journal of Psychiatry. 164:660-664 (1994).

24. S.B. Karch. The Pathology of Drug Abuse, 2nd Ed. CRC Press, Boca Raton, Florida. (1996).

25. L. Kambam, B. Mets, R.M. Hickman, P. Janicki, M.F.M. James, B. Fuller, and R.E. Kirsch. The effects of inhibition of plasma cholinesterase and hepatic microsomal enzyme activity on cocaine, benzoylecgonine, ecgonine methyl ester, and norcocaine blood levels in pigs. Journal of Laboratory Clinical Medicine. 120: 323-328 (1992).

26. M.R. Brzezinski, T.L. Abraham, C.L. Stone, R.A. Dean, and W.F. Bosron. Purification and characterization of a human liver cocaine carboxylesterase that catalyzes the production of benzoylecgonine and the formation of cocaethylene from alcohol and cocaine. Biochemistry and Pharmacology. 48: 1747-1755 (1994).

27. E.J. Cone, M. Hillsgrove, and W.D. Darwin. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. Clinical Chemistry. 40:1299-305 (1994).

28. D.S. Isenschmid, M.W. Fischman, R.W. Foltin, and Y.H. Caplan. Concentration of cocaine and metabolites in plasma of humans following intravenous administration and smoking of cocaine. Journal of Analytical Toxicology. 16:311-314 (1992).

29. L. Shuster. Pharmacogenetics of drugs of abuse. Annals of the New York Academy of Sciences. 562: 56-73 (1989).

30. A.M. Heith, C.R. Morse, T. Tsujita, S.A. Volpacelli, J.G. Flood and M. Laposata. Fatty acid ethyl ester synthase catalyzes the esterification of ethanol to cocaine. Biochem. Biophys. Res. Commun. 208:549-554 (1995).

31. J.S. Rose. Cocaethylene: a current understanding of the active metabolite of cocaine and ethanol. American Journal of Emergency Medicine. 12: 489-490 (1994).

32. B.W. Steele, E.S. Bandstra, N.C. WU, G.W. Hime and W.L. Hearn. *m*-Hydroxybenzoylecgonine: An important contributor to the

immunoreactivity in assays for benzoylecgonine in meconium. Journal of Analytical Toxicology. 17:348-352 (1993).

33. R.L. Andres and F.L. Jones. Social and illicit drug use in pregnancy. In: Maternal-Fetal Medicine: Principles and Practice, 3rd ed. R.K. Creasy and R. Resnik, ed. W.B. Saunders Company. Philadelphia, PA. 188-191 (1994).

34. M.A. Plessinger, and J.R. Woods, Jr. Maternal, placental and fetal pathophysiology of cocaine exposure during pregnancy. Clinical Obstetrics and Gynecology. 36: 267-278 (1993).

35. D.J. Ecobichon, and D.S. Stephens. Perinatal development of human blood esterases. Clinical Pharmacology and Therapeutics. 14: 41-47 (1972).

36. D.V. Parke. Development of detoxication mechanisms in the neonate. In: Toxicology and the Newborn. S. Kacew and M.J. Reasor ed. Elsevier Science Publishers, Amsterdam, The Netherlands. 1-31 (1984).

37. E. Jacqz-Aigrain and T. Cresteil. Cytrochrome P450-dependant metabolism of dextromethorphan: Fetal and adult studies. Developmental Pharmacology and Therapeutics. 18:161-168 (1992).

38. S. Schenker, Y. Yang, R.F. Johnson, J.W. Downing, R.S. Schenken, G.I. Henderson, and T.S. King. The transfer of cocaine and its metabolites across the term human placenta. Clinical Pharmacology and Therapeutics. 53:329-339 (1993).

39. G.M. Pacifici and R. Nottoli. Placental transfer of drugs administered to the mother. Clinical Pharmacokinetics. 28:235-269 (1995).

40. P.R. Mahone, K. Scott, G. Sleggs, T. D'Antoni, and J.R. Woods, Jr. Cocaine and metabolites in amniotic fluid may prolong fetal drug exposure. American Journal of Obstetrics and Gynecology. 171: 465-469 (1994).

41. J.M. Witkin and J.L. Katz. Preclinical assessment of cocaine toxicity: Mechanisms and pharmacotherapy. In: Acute Cocaine Intoxication: Current Methods of Treatment. NIDA Research Monograph, NIH publication No. 93-3498. 44-69 (1993).

42. C.H. Fox. Cocaine use in pregnancy. Journal of American Board Family Practice. 7: 225-228 (1994).

43. D. Mastrogiannis, G. Decavalas, U. Verma, and N. Tejani. Perinatal outcome after recent cocaine usage. Obstetrics & Gynecology. 78:8-11 (1990).

44. A. Oro and S. Dixon. Perinatal cocaine and methamphetamine exposure: Maternal and neonatal correlates. Journal of Pediatrics. 111:571-578 (1987).

45. I.J. Chasnoff, K.A. Burns, and W.J. Burns. Cocaine use in pregnancy: perinatal morbidity and mortality. Neurotoxicology and Teratology. 9: 291-293 (1987).

46. L. Slutsker. Risks associated with cocaine use during pregnancy. Obstetrics & Gynecology. 79: 778-789 (1992).

47. M.C. Spires, E.F. Gordon, M. Choudhuri, E. Maldonado, and R. Chan. Intracranial hemorrhage in a neonate following prenatal cocaine exposure. Pediatric Neurology. 5:324-326 (1989).

48. H.E. Hoyme, K.L. Jones, S.D. Dixon, T. Jewett, J.W. Hanson, L.K. Robinson, M.E. Msall, and J.E. Allanson. Prenatal cocaine exposure and fetal vascular disruption. Pediatrics. 85:743-747 (1990).

49. B.L. Tabor, T. Smith-Wallace, and M.L. Yonekura. Perinatal outcome associated with PCP versus cocaine use. American Journal of Alcohol and Drug Abuse. 16:337-348 (1990).

50. T.E. Yap, D. Diana, V. Herson, L. Chameides, and J.C. Rowe. Fetal myocardial calcification associated with maternal cocaine use. American Journal of Perinatology. 11: 179-183 (1994).

51. B.M. Lester, M.J. Corwin, C. Sepkoski, R. Seifer, M. Peucker, S. McLaughlin, and H.L. Golub. Neurobehavioral syndromes in cocaine-exposed newborn infants. Child Development. 62: 694-705 (1991).

52. J. Ho, E. Afshani, and F.B. Stapleton. Renal vascular abnormalities associated with prenatal cocaine exposure. Clinical Pediatrics. 33:155-156 (1994).

53. C.A. Chiriboga, D.A. Bateman, J.C. Brust, and W.A. Hauser. Neurologic findings in neonates with intrauterine cocaine exposure. Pediatric Neurology. 9:115-119 (1993).

54. C. Carraccio, J. Papadimitriou, and P. Feinberg. Subcutaneous fat necrosis of the newborn: link to maternal use of cocaine during pregnancy. Clinical Pediatrics. 33:317-318 (1994).

55. S.L. Davidson-Ward, D. Bautista, L. Chan, M. Derry, A. Lisbin, M.J. Durfee, K.S.C. Mills, and T.G. Keens. Sudden infant death syndrome in infants of substance-abusing mothers. The Journal of Pediatrics. 117: 876-881 (1990).

56. L. Ryan, S. Ehrlich, and L. Finnegan. Cocaine abuse in pregnancy: effects on the fetus and newborn. Neurotoxicology and Teratology. 9: 295-299 (1987).

57. A.J. Hadeed and S.R. Siegel. Maternal cocaine use during pregnancy: effect on the newborn infant. Pediatrics. 84: 205-210 (1989).

58. M.W. Church, B.A. Dintcheff, and P.K. Gessner. Dose dependent consequences of cocaine on pregnancy outcome in the Long-Evans rat. Neurotoxicology and Teratology. 10:51-57 (1987).

59. K. Kaye, L. Eklind, D. Goldberg, and A. Tytun. Birth outcomes for infants of drug abusing mothers. NY State Journal of Medicine. 89:256-261 (1989).

60. I.J. Chasnoff, D. Griffeth, S. MacGregor, K. Dirkes, and K.A. Burns. Temporal patterns of cocaine use in pregnancy. Journal of the American Medical Association. 261:1741-1744 (1989).

61. R.P. Kapur, C.M. Shaw, and T.H. Shepard. Brain hemorrhages in cocaine-exposed human fetuses. Teratology. 44:8-11 (1991).

62. J.J. Volpe. Effect of cocaine use on the fetus. The New England Journal of Medicine. 327:399-407 (1992).

63. M. Van-de-Bor, F.J. Walther, and M.E. Sims. Increased cerebral velocity in infants of mothers who abuse cocaine. Pediatrics. 85:733-736 (1990).

64. W.S. Webster and P.D.C. Brown-Woodman. Cocaine as a cause of congenital malformations of vascular origin: Experimental evidence in the rat. Teratology 41:689-697 (1990).

65. B.B. Little, L.M. Snell, V.R. Klein, and L.C. Gilstrap. Cocaine abuse during pregnancy: maternal and fetal implications. Obstetrics & Gynecology. 73:157-160 (1989).

66. I.J. Chasnoff, G.A. Chisum, and W.E. Kaplan. Maternal cocaine use and genitourinary tract malformations. Teratology 37:201-204 (1988).

67. G.F. Chavez, J. Mulinare, and J.F. Cordero. Maternal cocaine use during early pregnancy as a risk factor for congenital urogenital abnormalities. JAMA. 262:795-798 (1989).

68. N. Bingol, M. Fuchs, V. Diaz, R.K. Stone, and D.S. Gromisch. Teratogenicity of cocaine in humans. The Journal of Pediatrics. 110: 93-96 (1987).

69. B. Zuckerman and K. Bresnahan. Developmental and behavioral consequences of prenatal drug and alcohol exposure. Pediatr. Clin. North Am. 38:1387-1406 (1991).

70. J.D. Madden, T.F. Payne, and S. Miller. Maternal cocaine abuse and effect on the newborn. Pediatrics. 77: 209-211 (1986).

71. K.M. Gillogley, A.T. Evans, R.L. Hansen, S.J. Samuels, and K.K. Batra. The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening. American Journal of Obstetrics and Gynecology. 163:1535-1542 (1990).

72. B. Lutiger, K. Graham, T.R. Einarson, and G. Koren. Relationship between gestational cocaine use and pregnancy outcome: a meta-analysis. Teratology. 44:405-414 (1991).

73. C.M. Callahan, T.M. Grant, P. Phipps, G. Clark, A.H. Novack, A.P. Streissguth, and V.A. Raisys. Measurement of gestational cocaine exposure: sensitivity of infants' hair, meconium and urine. Journal of Pediatrics. 120: 763-768 (1992).

74. E.M. Ostrea, M. Brady, P.M. Parks, D.C. Asensio, and A. Naluz. Drug screening of meconium in infants of drug-dependent mothers: an alternative to urine testing. The Journal of Pediatrics. 115: 474-477 (1989).

75. B. Forslind. The growing anagen hair. In: Hair and Hair Diseases. C.E. Orfanos and R. Happle, eds. Springer-Verlag. New York. 70-97 (1990).

76. F.J.G. Ebling. The hormonal control of hair growth. In: Hair and Hair Diseases. C.E. Orfanos and R. Happle, eds. Springer-Verlag. New York. 267-299 (1990).

77. T. Inoue and S. Seta. Analysis of drugs in unconventional samples. Forensic Science Review. 4:89-106 (1992).

78. A.M. Baumgartner, P.F. Jones, W.A. Baumgartner and C.T. Black. Radioimmunoassay of hair for determining opiate-abuse histories. Journal of Nuclear Medicine. 20:748-759 (1979).

79. D.A. Kidwell and D.L. Blank. Mechanisms of incorporation of drugs into hair and the interpretation of hair analysis data. In: Hair Testing for Drugs of Abuse: International Research on Standards and Technology. E.J. Cone, M.J. Welch and M.B. Grigson-Babecki, eds. NIH Publication No. 95-3727. Rockville, MD. 19-90 (1995).

80. E.J. Cone, M.J. Hillsgrove, A.J. Jenkins, R.M. Kennan, and W.D. Darwin. Sweat testing for heroin, cocaine, and metabolites. Journal of Analytical Toxicology. 18: 298-305 (1994).

81. D. Garside and B.A. Goldberger. Determination of cocaine and opioids in hair. In: Drug Testing in Hair, P. Kintz, ed. CRC Press, Inc. Boca Raton. 151-180 (1996).

82. K. Graham, G. Koren, J. Klein. J. Schneiderman, and M. Greenwald. Determination of gestational cocaine exposure by hair analysis. Journal of the American Medical Association. 262: 3328-3330 (1989).

83. J. Klein, R. Forman, C. Eliopoulos, and G. Koren. A method for simultaneous measurement of cocaine and nicotine in neonatal hair. Therapeutic Drug Monitoring. 16: 67-70 (1993).

84. T.Grant, Z. Brown, C. Callahan, H. Barr, and A.P. Streissguth. Cocaine exposure during pregnancy: improving assessment with radioimmunoassay of maternal hair. Obstetrics & Gynecology. 83: 524-531 (1994).

85. P.R. Marques, A.S. Tippetts, and D.G. Branch. Cocaine in the hair of mother -infant pairs: Quantitative analysis and correlations with Urine measures and self report. American Journal of Drug and Alcohol Abuse. 19:159-173 (1993).

86. G.J. DiGregorio, E.J. Barbieri, A.P. Ferko, and E.K. Ruch. Prevalence of cocaethylene in the hair of pregnant women. Journal of Analytical Toxicology. 17:445-446 (1993).

87. G.J. DiGregorio, A.P. Ferko, E.J. Barbieri, E.K. Ruch, H. Chawla, D. Keohane, R. Rosenstock, and A. Aldano. Determination of cocaine usage in pregnant women by a urinary EMIT drug screen and GC-MS Analyses. Journal of Analytical Toxicology. 18: 247-250 (1994).

88. B.A Goldberger, Y.H. Caplan, T. Maguire, and E.J. Cone. Testing human hair for drugs of abuse. III. Identification of heroin and 6-acetylmorphine as indicators of heroin use. Journal of Analytical Toxicology. 15:226-240 (1991).

89. B. Rosner. Fundamentals of Biostatistics. Duxbury Press, Boston. (1982).

90. D.A. Frank, and B.S. Zukerman. Children exposed to cocaine prenatally: pieces of the puzzle. Neurotoxicology and Teratology. 15: 298-300 (1993).

91. L. Jain, W. Meyer, C. Moore, I. Tebbett, D. Gauthier, and D. Vidyasagar. Detection of fetal cocaine exposure by analysis of amniotic fluid. Obstetrics and Gynecology. 81: 787-790 (1993).

92. T. Inaba, D.J. Stewart, and W. Kalow. Metabolism of cocaine in man. Clinical Pharmacology and Therapy. 23:547-551 (1978).

93. K.L. Moore and T.V.N. Persuad. The Developing Human: Clinically Orientated Embryology. 5th Ed. W. B. Sanders Company. Philadelphia (1993).

94. W.A. Baumgartner, V.A. Hill, and W.H. Blahd. Hair analysis for drugs of abuse. Journal of Forensic Sciences. 34:1433-1453 (1989).

95. E.J. Cone, D. Yousefefnejad, W.D. Darwin, and T. Maguire. Testing human hair for drugs of abuse. II. Identification of unique cocaine metabolites in hair of drug abusers and evaluation of decontamination procedues. Journal of Analytical Toxicology. 15:250-255 (1991).

96. O.Q. Casanova, N. Lombardero, M. Behnke, F.D. Eyler, M. Conlon, and R.L. Bertholf. Detection of cocaine exposure in the neonate. Archives of Pathology and Laboratory Medicine. 118: 988-993 (1994).

97. C.M. Moore, S. Brown, A. Negrusz, i. Tebbett, W. Meyer, and L. Jain. Determination of cocaine and its major metabolite, benzoylecgonine, in amniotic fluid, umbilical cord blood, umbilical cord tissue, and neonatal urine: a case study. Journal of Analytical Toxicology. 17: 62 (1993).

98. R.A. Brace. Amniotic fluid dynamics. In: Maternal-Fetal Medicine: Principles and Practice, 3rd Ed. R.K. Creasy and R. Resnik, ed. W.B. Saunders Company. Philadelphia, PA. 106-114 (1994).

99. C. Lentner, ed. Geigy Scientific Tables. Ciba-Geigy Corp. West Caldwell, N.J. 197-202 (1981).

100. H. C. Atkninson, E. J. Begg, and B. A. Darlow. Drugs in Human Milk: Clinical pharmacokinetic considerations. Clinical Pharmacotherapy. 14:217-240 (1988).

101. P. H. Dickson, A. Lind, P. Studts, H. C. Nipper, M. Makoid, and D. Therkildsen. The routine analysis of breast milk for drugs of abuse in a clinical toxicology laboratory. Journal of Forensic Science. 39:207-214 (1994).

102. J.A. Madden and R. H. Powers. Effect of cocaine and cocaine metabolites on cerebral arteries in vitro. Life Sci. 47:1109-14 (1990).

103. B. Erickson, R.J. Konkol, and J.A. Madden. A comparison of the epileptogenic potential of cocaine and benzoylecgonine following intraventricular administration in nonanesthetized rats. FASEB J. 4:746A (1990).

104. B. Hanson and L. Moore. Recreational drug use by the breast feeding women, Part 1: Illicit drugs. Journal of Human Lactation. 5:178-180 (1989).

105. J.R. Woods and J.C. Glantz. Significance of amniotic fluid meconium. In: Maternal-Fetal Medicine: Principles and Practice, 3rd Ed. R.K. Creasy and R. Resnik, ed. W.B. Saunders Company. Philadelphia. 413-422 (1994).

106. P. Dahlem, H.U. Bucher, D. Cuedent, D. Mieth, and K. Gautschi. Prevalence of drugs in meconium. Monatsschr. Kinderheilkd. 141: 237-240 (1993).

107. S. Browne, C. Moore, A. Negrusz, I. Tebbett. R. Covert, and A. Dusick. Detection of cocaine, norcocaine, and cocaethylene in the meconium of premature neonates. Journal of Forensic Science. 39: 1515-1519 (1994).

108. D.E. Lewis, C.M. Moore, J.B. Leikin, and A. Koller. Meconium analysis for cocaine: a validation study and comparison with paired urine analysis. Journal of Analytical Toxicology. 19: 148-150 (1995).

109. F. Moriya, K. Chan, T.T. Noguchi and P.Y.K. Wu. Testing for drugs of abuse in meconium of newborn infants. Journal of Analytical Toxicology. 18: 41-45 (1994).

110. W.E. Wingert, M.S. Feldman, M.H. Kim, L. Noble, I. Hand, and J.J. Yoon. A comparison of meconium, maternal urine and neonatal urine for detection of maternal drug use during pregnancy. Journal of Forensic Sciences. 39: 150-158 (1994).

111. E.C. Maynard, L.P. Amoruso, and W. Oh. Meconium for drug testing. AJDC. 145: 650-652 (1991).

112. A.H.B. Wu, E. Forte, G. Casella, K. Sun, G. Hemphill, R. Foery, and H. Schanzenbach. CEDIA for screening drugs of abuse in urine and the effect of adulterants. Journal of Forensic Sciences. 40: 614-618 (1995).

113. Emit II, Cocaine Metabolite Assay Package Insert. Syva Company. San Jose, CA, (1993).

114. CEDIA, Cocaine Assay Package Insert. Boehringer Mannheim Corp. Indianapolis, (1994).

115. S. McCalla, H.L. Minkoff, J. Feldman, L. Glass, and G. Valencia. Predictors of cocaine use in pregnancy. Obstetrics & Gynecology. 79: 641-644 (1992).

BIOGRAPHICAL SKETCH

The author was born Ruth Ellen Ritch on January 12, 1965, in Atlanta, GA. After graduating from Duluth High School, she attended Oglethorpe University, located in Atlanta. While there she majored in biology with a minor in chemistry, as well as meeting her future husband, John Winecker. In 1987 she graduated *cum laude* and was awarded a bachelor of science degree.

After marrying John in April of 1988, the newlyweds moved to Gainesville, FL where the author pursued a master's degree in wildlife science. After two semesters, she decided that wildlife science was not the career for her and went to work at PCR, a chemical manufacturing plant in Gainesville.

In 1992, a senior chemist position at the University of Florida became available under Dr. Bertholf's supervision, which he offered the author, and she accepted. Concurrently, the author began part-time classwork in the interdisciplinary toxicology Ph.D. program. Two years later she resigned from her job and pursued her graduate degree full-time (although her husband objected, saying they would not be able to survive financially, he was wrong!).

Contingent upon graduation, the author has accepted a position as Deputy Chief Toxicologist with the Office of the Chief Medical Examiner in Chapel Hill, North Carolina.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Roger L. Bertholf, Chairman
Associate Professor of Pathology and Laboratory Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Bruce A. Goldberger
Assistant Professor of Pathology and Laboratory Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Catherine Hammett-Stabler
Assistant Professor of Pathology and Laboratory Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Ian Tebbett
Associate Professor of Pharmaceutics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Richard A. Yost
Professor of Chemistry

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree Doctor of Philosophy.

December, 1996

Dean, College of Medicine

Dean, Graduate School